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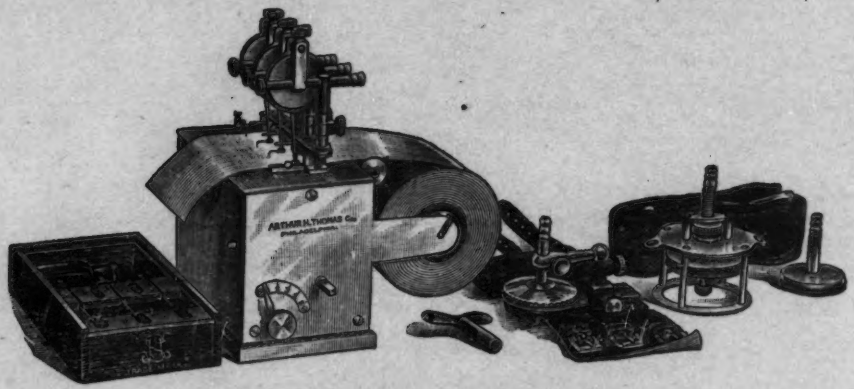

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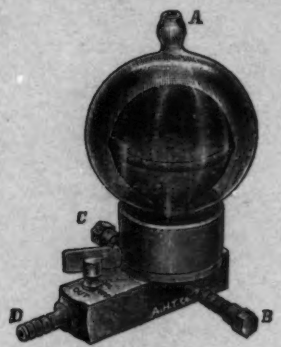

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THE RETURN OF UREA FROM THE KIDNEY TO THE BLOOD

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From the Laboratory of the Medical Division of Stanford University Medical School, San Francisco

Received for publication March 20, 1917

During unsuccessful attempts to measure the rate of flow of blood through the kidney, we found that the concentration of urea may be higher in the renal vein than in the renal artery. The rate of flow of blood through the kidney can be calculated if the urea concentration in the blood of the renal artery and vein is determined over a period during which the rate of flow of urine and the rate of urea excretion is estimated. But we found that we could not obtain these data without adopting measures designed to prevent or lessen vasoconstriction of the renal arteries. For after tying off one kidney and exposing the other, the manipulations required for the removal of blood from the renal vein were always attended by a cessation of the flow of urine which appeared to be due to vasoconstriction, since we were able in some instances to observe that the renal artery grew smaller. Renal vein blood obtained under these conditions contained more urea than the blood entering the kidney. The experiments cited in this paper extend and confirm this initial observation.

This additional urea found in the renal vein must have had its origin in some accumulation of urea within the kidney. The kidney contains more urea than other organs and is an exception to the rule of the approximately even distribution of urea throughout the tissues and fluids of the body (1). The microchemical work of Leschke (2) and of Oliver (3) demonstrates that the reason for this high urea content is the special concentration of urea in two separate situations in the kidney, in the cortex within the cells of the proximal convoluted tubules

and in the medulla in the urine lying within the collecting tubules. It is not possible to determine which of these stores of urea is the source of the urea returned to the renal blood, nor which contributes the greater part if the returned urea comes from both. We found that the medullary portion of the kidney contained somewhat more urea than the cortex, but on the other hand the cellular store in the cortex is in direct contact with the blood, while the urinary accumulation in the medulla is separated from the blood by a layer of renal cells.

But whatever the exact source of the urea added to the renal blood may be, the fact of its return from the kidney whenever the secretion of urine stops, throws some light on the mode of action of that force which enables the kidney to prepare a concentrated solution of urea such as the urine from a dilute solution of urea such as the blood. The accumulation of urea at certain locations within the kidney in much higher concentration than exists elsewhere in that organ must be accomplished by a force which is able to annul or overrule the physical laws governing the diffusion of urea. Such a force might act through the medium of some physical or chemical configuration which would remain passively operative even when the active functions of the kidney were in abeyance, just as the valve of a machine will continue to prevent the reflux of fluid when the power is shut off. But the immediate return of urea from the kidney to the blood indicates that this force has to be in continual operation to maintain the high urea concentration. When the kidney stops working this force relaxes its hold upon the heaped up urea, so that it again becomes subject to the laws of diffusion, and falls from the site of high concentration in the kidney to the lower levels of the blood, just as a weight held in the hand will fall to the ground when the grasp is relaxed.

THE RELIABILITY OF THE METHOD USED FOR DETERMINING THE UREA CONTENT OF THE BLOOD

Triplicate determinations were made on each blood except in a few instances in which only duplicates were obtained because of accident to one of the samples or failure to obtain enough blood.

From such material an expression of the probable error in the measurements might have been obtained by finding the standard deviation for each set of triplicate or duplicate results, and multiplying the average of these standard deviations by 0.67. This procedure however is not only cumbersome but has been shown by Otis (4) to give a value

less than the true probable error. He has demonstrated that the median of all the differences between triplicate or duplicate measurements divided by the square root of 2 gives the probable error of a single determination. The probable error of the average of three determinations is this probable error divided by the square root of 3.

The differences between repeated determinations on a blood with a high urea content were no greater than those found when the urea content of the blood was low. All such differences are therefore directly comparable. There were in all 168 differences. The median of this series was 0.9 mgm. Applying the formula we obtain a probable error of 0.64 mgm. for single determinations and 0.37 mgm. for the averages of three determinations such as are given in our tables. In other words, in half of our figures the quantity recorded, e.g., 100 mgm., might have been any value between 100.37 mgm. and 99.63 mgm. In the other half of our figures, the error is greater than 0.37 mgm. Mr. Otis pointed out to us that if the frequency distribution of errors may be assumed in this case to be "normal," that is, in accordance with the law of the distribution of errors, then theoretically the errors will be less than twice the probable error in 82 per cent of the cases, less than three times the probable error in 95 per cent, and less than four times the probable error in slightly over 99 per cent of cases. In only one case in a hundred, therefore, will the error reach 1.5 mgm.

The urease method of Marshall was used, carried out in much the same way as is recommended by Van Slyke and Cullen. The quantity of blood taken for each determination was 1 cc. measured with an Ostwald pipette. Care was taken that the bloods whose urea content was to be compared were treated in an exactly similar manner as regards the amount of soy bean extract added, and the length of time of incubation and aeration. The acid was measured with an automatic pipette. In titration those refinements were used which were introduced by Barnett (5) in connection with his method for determining small quantities of ammonia.

An increase in the urea content of blood from the renal vein at a time when the secretion of urine had stopped, was observed in a number of the unsuccessful attempts to measure the rate of flow of blood through the kidney referred to at the commencement of this paper. Those figures are not given since the determinations were carried out on such small quantities of blood that the reliability of the method

was considerably less than when the larger quantities available in the experiments cited here were used.

The animals used were rabbits. In some cases urea was given by stomach tube before operation in order to increase the urea content of the blood.

THE DECREASE IN THE UREA CONTENT OF BLOOD FROM THE RENAL VEIN WHEN PRECAUTIONS ARE TAKEN TO DISTURB THE FUNCTION OF THE KIDNEY AS LITTLE AS POSSIBLE

As soon as the animal was fully under the influence of ether, the left kidney was fully exposed through an incision in the flank, the renal vein cut with scissors and the blood collected in a vessel containing a little powdered oxalate.

In two rabbits a direct comparison was made between the urea content of the renal vein blood and the blood of the renal artery. Two ligatures were placed in position round the renal vein, the one next the vena cava tied, the swollen vein snipped with scissors, and after enough blood had collected the one next the hilus of the kidney was tied. Immediately afterwards the renal artery was cut. A decrease was found in the venous blood, 12 and 39 mgm., as against 15 and 41 mgm. in the arterial. The venous blood was slightly more concentrated as judged by the relative volumes occupied by red blood cells and plasma, but not to such a degree as appreciably to affect the urea content.

Such direct comparisons between blood from the renal vein and artery have the disdavantage of involving a cessation of the flow of blood through the kidney and therefore carry with them a tendency to interference with kidney function. Further, even such brief manipulations as are required in clamping or ligaturing the renal vein in two places are apt to induce a constriction of the renal artery. In our other experiments we have therefore taken the blood of the jugular vein as representing blood from the renal artery so far as its urea content is concerned. In a few experiments blood from the femoral artery was used. That this is justifiable is shown by the result of comparisons of the urea content of blood from the renal artery with the urea content of blood from the jugular and carotid. Such differences as are recorded are not significant. (table 1.)

In twelve rabbits the renal vein blood was compared with the jugular. The jugular was first bared, the kidney quickly exposed and the

renal vein snipped with scissors. Immediately thereafter, without waiting to tie the renal vein, the jugular was cut. The whole procedure did not take more than one or two minutes, and there was no mechanical interference with the circulation through the kidney. The results are given in table 2.

TABLE 1

Comparison of the urea content of blood from the renal artery, jugular vein and carotid artery

RENAL ARTERY	JUGULAR VEIN	CAROTID ARTERY
mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*
40.47	40.00	
21.00	22.37	
24.50		24.47
83.35	83.90	
19.57	19.74	
	24.75	25.47

TABLE 2

Comparison of the urea content of blood from the renal vein and the jugular vein when the blood was taken quickly

RENAL VEIN	JUGULAR VEIN	LESS IN RENAL VEIN	MORE IN RENAL VEIN
mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
47	57	10	
27	27		
180	189	9	
97	102	5	
22	23	1	
42	53	12	
184	196	12	
212	223	11	
197	196		1
38	38		
139	149	10	
211	216	5	

There can be no question from these figures that in some of these cases the kidney must still have continued to remove urea from the blood passing through it. In seven out of the twelve experiments the decrease in the urea content of the venous blood is considerably greater than could be accounted for on the basis of technical error.

A DECREASE IN THE UREA CONTENT OF THE BLOOD OF THE LEFT RENAL VEIN WHEN THE BLOOD IS TAKEN QUICKLY FOLLOWED BY AN INCREASE IN THE UREA CONTENT OF THE BLOOD OF THE RIGHT RENAL VEIN OBSERVED IN THE SAME ANIMAL AFTER A PERIOD OF OPERATIVE MANIPULATION DURING WHICH THE SECRETION OF URINE CEASED

In seven rabbits, blood was obtained quickly in the manner already described from the left renal and jugular veins. The vessels of the left kidney were then clamped and ligatured. The bladder was opened and a catheter inserted into the right ureter. This was done in order to make sure that the secretion of urine had stopped. We did not

TABLE 3

Comparison of the urea content of blood from the left renal vein and the jugular vein when the blood was taken quickly and comparison of the urea content of blood from the right renal vein and the jugular vein when the secretion of urine had stopped

RABBIT NO.	BLOOD TAKEN QUICKLY				BLOOD TAKEN WHEN THE SECRETION OF URINE HAD STOPPED			
	Left renal vein	Jugular vein	Difference		Right renal vein	Jugular vein	Difference	
			More	Less			More	Less
	mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*
11	169	178		9	194	188	6	
15	88	93		5	100	100		
38	92	96		4	105	105		
39	180	187		7	201	189	12	
40	130	129		1	136	131	5	
41	130	140		10	145	139	6	
42	77	78	1		87	87		

obtain urine from the catheter in any of these animals. The right kidney was then exposed and blood collected from the right renal vein and immediately afterwards from the jugular. In a few cases the comparison was made with blood from the femoral artery, as enough blood was not obtainable from the jugular.

The urea content of the renal vein could thus be compared with that of blood corresponding in urea content to the blood sent to the kidney in the renal artery under two conditions, first at a time and under circumstances in which the kidney might still be functioning, and secondly at a time when we had evidence that kidney function had ceased. The results are given in table 3.

It will be noted that there is in general a decrease in the urea content of the renal vein when the blood is taken quickly, and an increase when the blood is taken at a time when no urine is being secreted. That there should be considerable variation in the amount of the decrease or increase is to be expected, since neither the degree of kidney activity nor the time at which that activity ceased was known or exactly controlled. But that there should be in any of these cases a clear decrease in the urea content of the renal vein blood can only be accounted for by the passage of urea from the blood into the kidney. Similarly a definite increase under other conditions is only to be explained by the return of urea from the kidney to the blood.

SUMMARY

When blood is taken from the renal vein so as to disturb the function of the kidney as little as possible, it usually contains less urea than the blood sent to the kidney in the renal artery. This is explained by the passage of urea from the blood to the kidney.

When blood is taken from the renal vein at a time when the secretion of urine has stopped, it usually contains more urea than the blood sent to the kidney in the renal artery. This is explained by the passage of urea from the kidney to the blood.

The return of urea from the kidney to the blood whenever the kidney ceases to secrete urine is taken as indicating that the force or forces which store urea in high concentration within the kidney must remain in continued operation to hold that accumulated urea, and do not act through the medium of any physical or chemical mechanism which would passively maintain its hold upon the urea even when the active concentration of new urea from the blood had ceased.

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(4) Otis: (Stanford University). To be published in the near future.
(5) Barnett: Journ. Biol. Chem., 1917, xxix, 459.

ADDENDUM

Some time after we had sent our manuscript for publication a paper by Cushny (Journ. Physiol., 1917, li, 36) reached us, which contains data demonstrating in another way the return of urea from the kidney to the blood after the activity

of the organ ceases. One kidney was removed while urine was being secreted, and its urea content per gram of tissue determined. At the same time the cord was cut in the cervical region so that a pronounced drop in blood pressure was produced and urine secretion stopped. After an interval of one to one and one-half hours the remaining kidney was removed. It was found to contain less urea than the kidney removed while still active.

A COMPARISON OF THE EFFECTS OF BREAKFAST, OF NO BREAKFAST AND OF CAFFEINE ON WORK IN AN ATHLETE AND A NON-ATHLETE

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From the Physiological Laboratory of the University of Kansas

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INTRODUCTION

The results pertaining to this investigation were obtained in 1912 and 1913, and are reported under two sections. The first section deals with experiments secured with a modified Lombard type of ergograph; the second section deals with tests obtained with an ergometer.

The experiments were conducted on two men in perfect health but of very different physical training.

Subject "B," the athlete, was 5 feet, 8 inches in height, weighed 196 pounds, was 29 years of age and an instructor in physical education. During the two years preceding these tests, in addition to his duties, he had been accustomed to take daily exercise, especially of the arms, chest and back muscles.

Subject "A," the non-athlete, was 5 feet in height, weighed 140 pounds and was 26 years of age. Before beginning these tests he had taken no special physical exercise.

The object of the experiment was to compare the pulse rate, blood pressure, ergographic and ergometer work in both men under the following conditions: of certain doses of caffeine without breakfast, breakfast without caffeine, neither breakfast nor caffeine, and of different intervals of time following the partaking of caffeine or breakfast.

The literature bearing directly on this problem is limited. The references will be confined to reports that may aid to a better understanding of the subject. The ergographic work, calculated from the cyclometer that recorded the sum of the heights of the contractions multiplied by the weight lifted, was performed by the flexor muscles of the second finger of the right hand, lifting a weight of 5 kgm. every two seconds. To prevent the neighboring flexors from participating, the first and third fingers were placed in an adapted clamp, and the

hand held securely in pronation to the ergographic support by means of a narrow non-elastic bandage, placed back of the metacarpal-phalangeal joints. With this arrangement the subjects were able to work more than an hour without discomfort or interference with the circulation.

The experiments were performed in the morning between 8 and 10 o'clock, and under similar conditions. Breakfast at 7.15 a.m., for each man consisted of one soft boiled egg, 2 ounces wheat bread and $\frac{3}{4}$ ounce of butter. For the experiments with caffeine, no breakfast was taken, instead 7 ounces of coca-cola,[1] containing a total of 1.42 grains of caffeine, being the average amount in a strong cup of coffee or of tea. The experiments with caffeine alternated with those obtained either with or without breakfast. The athlete did not drink coffee and the non-athlete had taken it for breakfast, but several weeks before and during the time that the experiments were in progress, of course, both of the subjects gave up the use of articles of diet that had caffeine in them.

Records were kept of the hours and conditions of sleep, of the pulse and systolic blood pressure on rising, before and after exercise, and the intervals elapsing between exercise and partaking of food or of caffeine. The pulse and blood pressure were secured with a Tycos sphygmomanometer while the subject was seated.

SECTION I. ERGOGRAPHIC WORK WITH FLEXOR MUSCLES

Preliminary to the main experiments, the flexor muscles in both subjects were daily exercised on the ergograph under like conditions. After six weeks of training the results became more constant, and for practical purposes the muscles were considered in training. Before that time, therefore, the muscles were considered untrained. Part 1 deals with the average results of the untrained; part 2, with those of the more trained flexors.

Part 1. For purposes of comparison, only the average of all the results observed were tabulated in table 1. A study of this table reveals that at the beginning and after eating breakfast, "A" could contract the untrained flexors two hundred and seventy-nine times until utterly fatigued in nine minutes and eighteen seconds, doing 12.65

[1] Analysis of coca-cola syrup: Sugar 53 per cent, caffeine 1.42 per cent, water 44 per cent, citric and phosphoric glycerine and alcohol qualitative test. One ounce of syrup equals about 7 fluid ounces of coca-cola.

kgm. of work; but by the end of the month he actually trebled these figures. On the other hand, it is seen that from the first "B" did one and one-half times as much work: 18.75 kgm. of work in eight minutes, fifty-five seconds, and in less time than "A" did his, and that he more than trebled his power for work during the month's training. Now instead of doing one and one-half times as much, he did one and two-thirds times as much as did "A," in one and one-fifth the time.

TABLE 1

Average results of experiments with untrained flexors on the ergograph. (a) The first and twenty-fourth test. (b) Average of eight experiments after eating breakfast. (c) Without breakfast. (d) After taking caffeine.

	DATE 1912	CONDITION	SUBJECT	DURATION OF WORK IN MINUTES	NUMBER OF CONTRACTIONS	DISTANCE LIFTED IN CENTIMETERS	WORK IN KILOGRAM METERS
(a)	November 19	Breakfast	A	9′ 18″	279	253	12.65
	December 20	Breakfast	A	26′ 54″	798	726	36.30
(a)	November 19	Breakfast	B	8′ 55″	273	375	18.75
	December 20	Breakfast	B	30′ 18″	918	1,205	60.25
(b)		Breakfast	A	14′ 17″	402	377	18.85
(b)		Breakfast	B	15′ 24″	462	733	36.65
(c)		No breakfast	A	12′ 00	360	357	17.75
(c)		No breakfast	B	15′ 03″	398	665	35.25
(d)		Caffeine	A	25′ 18	759	939	46.95
(d)		Caffeine	B	38′ 43″	1,154	1,421	71.05

The average hours sleep for both subjects = $7\frac{1}{2}$.

The lapse of time between breakfast and caffeine and exercise = 1 hr.

The average room temperature = 59° F.

The number of contractions until fatigued = until unable to lift the 5 kgm. weight.

These experiments were begun November 19 and ended December 20, 1912.

Comparing the amount of work done, without breakfast, one hour after breakfast, and also after a dose of caffeine, it was noticed that both subjects did almost as much without, and in practically the same time, as after having eaten breakfast. "B" moreover, felt better than when working after having eaten the meal.

When both subjects took a dose of 1.42 grains of caffeine, "A" was able to do 46.97 kgm. of work which was two and one-half times as much work, and "B" 71.05 kgm., which was twice as much work

as when working one hour after eating breakfast. The after effect for both, but more observable in "B," was a heightened sense of irritability and weariness not noticed except when working after taking caffeine. The reason that the same dose of caffeine stimulated the working power of the athlete less than it did the non-athlete was probably because the athlete is the larger and heavier man. The dose per kilo weight was less, therefore, for him than it was for "A."

A consideration of this set of experiments demonstrates that the flexors and probably other untrained muscles in an athlete, are more efficient and more readily trained, than are the untrained muscles in a non-athlete.

Part 2: Ergographic results with flexor muscles in training. After six weeks of training the muscles, the results became more constant, and the second set of experiments was begun, and continued for two months. The object of this set of experiments was to compare the effects of no breakfast, of breakfast one hour and one and one-half hours before work. The average results are tabulated in table 2. It is there shown that when working without having eaten breakfast, "B," the athlete, continued as before (table 1) to do twice as much work in one and one-half the time, as did "A." But when working one hour or one and one-half hours, after having eaten breakfast, although both of the men increased their power for work enormously, they did more after eating than when not eating breakfast, and more in one hour, and in less time, than in one and one-half hours after eating breakfast. Nevertheless "B" no longer did twice as much work as "A" but only one and one-half as much. It may be that the greater increase in power in "A" is due to the fact that he attained his maximum power more gradually than "B" did his. The ergographic work had practically no effect either on the pulse or blood pressure. The normal blood pressure was much higher in "B" than it was in "A" but there was little difference in their pulse rate. It appears, and this agrees with Lombard's (1) results, that exercise of this character can be performed better one hour than one and one-half hours after eating breakfast.

SECTION II

The influence of neither breakfast nor caffeine, breakfast without caffeine, and caffeine on ergometer muscle work in a trained athlete and a non-athlete. The ergometer consists of an adjustable grip bar, connected through two pulleys to a weight of 25 kgm. The recorder,

similar to the one attached to the ergograph consists of an endless tape, 1.5 cm. wide and 500 cm. in length that passes around two pulleys, each 50 cm. in circumference. A cyclometer attached to one of the pulley records, therefore, 50 cm. for each revolution. These are noted, and thus the whole height of contraction can be directly ascertained, and the work in kilograms determined.

In conducting the experiment, the subject stands on a line, a definite distance from the bar. The bar has been properly adjusted to the height of the subject, so that with arms fully extended he is able to grip it firm-

TABLE 2

Average results of flexor muscles in training on ergograph, no breakfast (A_1 B_1) *one hour* (A_2 B_2), *and one and one-half hours* (A_3 B_3) *after eating breakfast*

SUBJECT	CONDITION	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	CENTIMETERS LIFTED	NUMBER OF CONTRACTIONS	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			hours								
A_1	No breakfast	67		33′29″	1,194	994	59.7	69	71	108	109
A_2	Breakfast	72	1	49′00″	1,990	1,470	99.5	71	70	109	109
A_3	Breakfast	74	1½	40′	1,754	1,186	87.7	71	70	107	107
B_1	No breakfast	67		50′	2,487	1,494	124.3	70	71	129	130
B_2	Breakfast	72	1	60′	3,150	1,800	157.5	75	73	128	130
B_3	Breakfast	73	1½	65′	2,571	1,950	128.5	75	72	128	128

The experiments were begun January 15 and continued until March 15, 1912, the different tests alternating with each other during that time.

ly. The bar is lowered by the contraction of the muscles of the chest and arms as far as possible without stooping or raising the heels. In this way the 25 kgm. weight is raised a definite distance, and this distance is read from the recorder. The contractions were repeated every three seconds to the beat of a metronome and in every case were the result of maximum effort, and continued until the weight could no longer be lifted. The three seconds time was adopted because, after the muscles were in training, it proved to be the least time in which the rested muscles could complete a full contraction. In view of the fact that the experiments with the flexor muscles of the middle finger which, when worked on the ergograph until utterly fatigued, had, if any, but a slight effect on the pulse rate and blood pressure, it was

decided to repeat the experiments discussed in Section I with a set of muscles employed under ordinary conditions in hard labor. For this purpose the ergometer is admirably suited.

The experiments were begun March 15, 1913, and continued until June 6, 1913. Records of the first month's preliminary work were not kept. Both subjects exercised their muscles daily by repeating on the ergometer the order of the work that was to be followed, as soon as the muscles in both subjects were given equal training and had attained a certain degree of efficiency and uniformity of results. The tests were made as nearly as possible under similar conditions, and by alternating those with no breakfast, with those performed after eating breakfast or after taking caffeine.

Effect of no breakfast. Table 3 shows the average of ten experiments performed by each subject without eating breakfast.

TABLE 3

An average of ten ergometer records without breakfast

SUBJECT	NUMBER OF OBSERVATIONS	WEATHER CONDITIONS	ROOM TEMPERATURE	DURATION OF WORK	NUMBER OF CONTRACTIONS	CENTIMETERS LIFTED	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
A	10	Cold, stormy	66	2′ 18″	46	3,824	956	70	88	107	130
B	10	Cold, stormy	68	3′ 31″	70	8,305	2,074.2	73	102	128	150

From a study of the tabulated results it is learned that "B" did 2074 kgm. of work in three minutes, thirty-one seconds, and "A" 956 kgm. in two minutes, eighteen seconds. Therefore, "B" did more than twice as much work and in one and one-half the time, that "A" was able to accomplish his, but his blood pressure rose no higher above the level, while his pulse was one and one-half times more rapid than that of "A."

Effect of length of time after eating breakfast on work. The object of this series of experiments was to ascertain if the length of the interval following breakfast and the beginning of exercise influenced the ergometer work, the pulse and blood pressure.

Owing to the lack of time, only the one, one and one-half, two, and two and one-half hour intervals were tested.

Table 4 records four average tests of the sixteen carried out by each subject. It shows that the duration and power for muscular work in both men gradually increased as the interval following the meal lengthened from one up to two and one-half hours. At that time "B" did one and five-eighths as much work in less than one and one-half the time that "A" did it; also, that "B" accomplished as much without as when working one and one-half hours after eating breakfast, and that "A" did more work after eating than without breakfast.

TABLE 4

Ergometer records showing the effect of different intervals between breakfast and work

SUBJECT	NUMBER OF OBSERVATIONS	WEATHER CONDITIONS	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	CENTIMETERS LIFTED	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
				hours								
A	4	Generally stormy with snow, the temperature ranged from 25° to 66° F. with the lower temperature predominating.	76	1	2′ 40″	53	4,651	1,162.7	72	88	110	130
	5		74	1½	3′ 05″	62	5,508	1,377.0	72	84	100	128
	4		71	2	3′ 08″	63	5,599	1,399.0	68	78	106	126
	3		75	2½	3′ 20″	67	6,670	1,667.5	76	88	108	128
B	4		68	1	3′ 10″	63	7,986	1,996.5	76	116	128	158
	5		62	1½	3′ 35″	72	8,078	2,019.5	68	108	132	152
	4		60	2	3′ 55″	78	9,547	2,386.7	68	100	128	148
	3		60	2½	4′ 50″	96	10,710	2,677.5	72	92	134	154

The increase in pulse rate in "B" above normal was, as a rule in every case, more than double the increase in "A." In both subjects, however, the acceleration was less two and one-half hours after doing the greatest amount of work, than one hour following the meal.

The rise in blood pressure above the normal level was practically the same in both men, notwithstanding the athlete did far more work.

It was interesting to learn that with the ergograph the maximum

work was done one hour after, while with the ergometer the power for muscular work was greater two and one-half hours after eating breakfast.

The gradual effect of different doses of caffeine without either breakfast or work on the pulse rate and the blood pressure. The average of eight

TABLE 5

Average effect of different doses of caffeine without either breakfast or work on pulse and blood pressure, (A_1 B_1) dose = 1.42 grains, (A_2 B_2) dose = 2.24 grains of caffeine

SUBJECT	CONDITION		LAPSE OF TIME	PULSE	BLOOD PRESSURE
A_1	No breakfast, record taken	8.05		72	110
	Drank 1.42 grains caffeine	8.10			
	Record taken at	8.30	20′	71	119
	Record taken at	8.40	30′	73	122
	Record taken at	8.55	45′	76	124
	Record taken at	9.10	60′	76	126
	Record taken at	9.40	90′	75	125
	Record taken at	11.10	3 hr.	72	118
A_2	No breakfast, record taken	8.05		72	110
	Drank 2.24 grains caffeine	8.10			
	Record taken at	8.30	20′	80	124
	Record taken at	8.40	30′	68	128
B_1	No breakfast, record taken	8.05		68	128
	Drank 1.42 grains caffeine	8.10			
	Record taken at	8.30	20′	71	144
	Record taken at	8.40	30′	75	148
	Record taken at	8.55	45′	73	146
	Record taken at	9.10	60′	74	144
	Record taken at	9.40	90′		
	Record taken at	11.10	3 hr.	68	141
B_2	No breakfast, record taken	8.05		68	128
	Drank 2.24 grains caffeine	8.10			
	Record taken at	8.30	20′	71	144
	Record taken at	8.40	30′	84	151

tests, showing the effects of 1.42 and 2.24 grains of caffeine on the heart rate and the blood pressure are recorded in table 5. As will be shown later, the 2.24 grain dose for the athlete is equal per kilo body weight to 1.42 grain for the non-athlete. The first effect of 1.42 grain in "A" was a slowing of the pulse of twenty minutes duration, then a

gradual acceleration of four beats per minute during the next hour, and a return to the normal rate within three hours. With the larger dose there was no initial fall, but a rapid acceleration of ten beats per minute during the first twenty minutes and a slowing below the normal rate in thirty minutes.

In "B" the initial fall was absent, but both of the doses caused a gradual acceleration in the heart rate that appeared more promptly after taking the stronger dose, but after taking the weaker dose persisted for three hours, before the normal rate was again attained.

TABLE 6

Effect of taking caffeine at different intervals before work

SUBJECT	NUMBER OF OBSERVATIONS	AMOUNT OF CAFFEINE	ROOM TEMPERATURE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAM METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
		grains	°F									
A	8	1.42	66	20′	4′ 27″	87	7,486	1,871	72	106	118	135
	12		66	30′	3′ 33″	71	8,511	2,138	72	110	118	138
	8		70	45′	4′ 23″	88	10,204	2,538	76	110	124	138
	8		70	1 hr.	5′ 34″	111	12,101	3,025	76	117	126	139
	8		66	3 hr.	9′ 10″	183	19,380	4,827	72	128	118	148
B	8		66	20′	5′ 27″	109	13,287	3,321	71	109	144	161
	10		70	30′	4′ 53″	97	13,906	3,476	72	112	142	160
	8		70	45′	6′ 40″	133	14,586	3,646	70	110	146	164
	8		72	1 hr.	5′ 42″	114	13,127	3,381	74	112	143	163
	8		68	3 hr.	5′ 38″	112	12,209	3,052	78	104	142	158

The blood pressure rose about 20 mm. Hg above the level within one hour in both men, after taking either one of the doses, but after the weaker dose was taken, the normal level was not reached in either of the men within three hours.

The effect of taking caffeine from twenty to one hundred and eighty minutes before beginning ergometer work. From an inspection of table 6 where the average results are tabulated, it is seen, that in "A" the power for work steadily increased as the interval between taking the drug and beginning work increased, from twenty minutes up to three hours. Three hours after taking the 1.42 grains of caffeine, he was able to do 4827 kgm. of work in nine minutes, ten seconds, which was two and one-half times the work he was able to do twenty minutes

after having taken the drug. In fact this dose seemed to exert its greatest effect in three hours after it was taken by "A," while in "B," the athlete, its optimum influence was manifested three-quarters of an hour after the drug was taken, but he did only one-eleventh more work at that time than he did twenty minutes after taking the drug. In both men the blood pressure did not rise much higher than was usual after work without the drug, except that in "A," at the three-hour interval, it rose 13 mm. higher than it did twenty minutes after the dose was taken, and his pulse rate also increased with those intervals from thirty-four to forty-six beats per minute.

In comparing the results obtained at the optimum period that is two and one-half hours after eating breakfast (table 3), with those secured at the optimum interval, or three hours for "A" and three-quarter hours for "B," after taking 1.42 grain of caffeine (table 6), the following important facts are brought to our notice: that at those specified periods "A" did three times as much work, his pulse was almost five times as much accelerated, and his blood pressure rose one and one-half as high above the normal level after taking the 1.42 grain, as was possible two and one-half hours after eating breakfast. "B" did not do quite one and one-half as much work, his pulse increased twice the rate and his blood pressure was practically the same three-quarters of an hour after taking 1.42 grain of caffeine as two and one-half hours after eating his breakfast. Consequently this dose had a more prolonged and much greater effect three hours after taking the drug on the force, rate and power of muscular contraction, and upon the cardiac tissue in "A," the lighter weight subject, than it did at any time in "B," the heavier man, for whom it consequently was a weaker dose per kilo of his body weight.

The effect of different doses of caffeine on work. It became of interest to ascertain the effect of larger doses of caffeine, and for that purpose a series of experiments was conducted with 1.42 grain, 2.84 grains and 3.58 grains of caffeine, allowing thirty minutes in each case before the work was begun. This interval was decided upon in order to economize time and because the results for this interval had been quite constant for both subjects. The average results of these experiments are recorded in table 7. They show that endurance and power for work do not keep pace with increase of dosage, but that both subjects did their maximum work after taking the medium dose of 2.24 grains at the thirty minute interval chosen for comparison for these tests. With that dose they could lift the weight a greater number of times,

with greater force, and work longer before fatigued than they could at that interval after taking either of the other doses. "B" did 5429 kgm. work in nine minutes, fifty-four seconds, lifting the 25 kgm. one hundred and ninety-eight times. "A" did 2680 kgm. work in five minutes, fourteen seconds, lifting the 25 kgm. one hundred and four times. With the strongest dose of 3.58 grains, both subjects did less work than they did with the medium dose. In fact "A" did even less after taking the strongest and "B" only one-seventh more than after taking the weakest dose of 1.42 grain. It is evident that the strongest dose proved more depressing to both men than did the medium, and far more so to "A," the lighter weight man, than to "B" the heavier weight man.

TABLE 7

Average effect of different doses of caffeine on work

SUBJECT	NUMBER OF OBSERVATIONS	ROOM TEMPERATURE	AMOUNT OF CAFFEINE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
		°F	*grains*									
A	12	66	1.42	30′	3′ 33″	71	8,511	2,138	72	110	118	138
B	10	66	1.42	30′	4′ 53″	97	13,906	3,476	72	112	142	160
A	8	70	2.24	30′	5′ 14″	104	10,700	2,680	69	119	125	137
B	8	70	2.24	30′	9′ 54″	198	21,716	5,429	71	116	145	165
A	6	70	3.58	30′	3′ 27″	69	7,109	1,777	71	118	124	131
B	3	70	3.58	30′	7′ 20″	147	15,728	3,932	78	116	136	156

The strongest dose of 3.58 grains greatly accelerated the pulse rate, but depressed the blood pressure below the normal level after work in "A," while it had only a little more influence on the pulse and practically no more on the blood pressure in "B" than the medium dose had on these activities.

The effect of caffeine when the dose is taken per kilo body weight. Two sets of experiments were undertaken for the purpose of ascertaining the effects of caffeine thirty minutes after each subject received equal amounts of caffeine per kilo body weight. In the first set of experiments "A," who weighed 66.6 kilos, took 1.42 grains, and "B," whose weight was 93.3 kilos took 2.24 grains of the drug. Each subject was then receiving practically 0.21 grain per 9.3 kilo of his weight. In the

second tests, subject "A" took 2.24 grains and "B" 3.58 grains. The results obtained from these experiments, and which are summarized in table 8 add another viewpoint to the facts obtained from the experiments that dealt with equal doses of caffeine for each subject, without respect to their weight.

At the thirty minute interval after each subject received the weaker dose of 0.21 grain of caffeine per kilo of his body weight, "B" was able to do two and one-half times as much work, and work almost three times as long before becoming fatigued, as was "A." His heart rate at the same time was only seven more beats per minute than was that in

TABLE 8

Effect of caffeine when given per kilo body weight

SUBJECT	NUMBER OF OBSERVATIONS	ROOM TEMPERATURE	AMOUNT OF CAFFEINE	LAPSE OF TIME BEFORE WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			grains									
1 A	12	66	1.42	30′	3′ 33″	71	8,511	2,138	72	110	118	138
1 B	8	70	2.24	30′	9′ 54″	198	21,716	5,429	71	116	145	165
2 A	8	70	2.24	30′	5′ 14″	104	10,700	2,680	69	119	125	137
2 B	3	70	3.58	30′	7′ 20″	147	15,728	3,932	78	116	136	156

1 = 0.2 grains of caffeine per 9.3 kilo body weight = A 1.42 grains, B 2.24 grains.

2 = 0.2 grains of caffeine per 5.9 kilo body weight = A 2.24 grains, B 3.58 grains.

These experiments were begun March 28 and ended June 6, 1913.

"A," while the increase in blood pressure above the normal level was the same in both. Thirty minutes after each subject received the stronger dose of 0.21 grain per 5.9 kilo of their body weight, "A" did one-fourth more work, and worked two-thirds longer before becoming fatigued than he did with the weaker dose. "B," however, was able to do only about two-thirds as much work, and work about two-thirds as long as he could with the weaker dose. Therefore "B" now did only one and one-half as much work and worked only one and two-thirds as long as "A" before becoming fatigued. His pulse rate was

accelerated thirty-eight and "A's" fifty beats per minute. On the other hand his blood pressure was 20, and "A's" only 12 mm. Hg above the normal level.

Comparing the results obtained from these two doses of caffeine, the fact is brought out that in "B," the heavier subject, the weaker dose of 0.21 grain per 9.3 kilo body weight was more stimulating to the muscular and cardiac activity than was the stronger dose of 0.21 grain per 5.9 kilo per body weight. On the other hand in "A" the stronger dose of 0.21 grain per 5.9 kilo body weight proved the more stimulating. Therefore we conclude that doses given per kilo weight exert for each individual a specific effect, and that at the same interval after taking the drug the effect may be different for different individuals.

The after-effect of caffeine. It became of interest to ascertain whether caffeine exerted a prolonged influence on the system. For this purpose twelve experiments were performed consisting of three sets of four each; with 1.42 grain, 2.84 grains caffeine, and control tests respectively. For the control tests no breakfast was eaten. Each experiment involved five observations on the effect of ergometer work, namely, at 8.20 and 9.20 a. m., and at 4.20 and 8.20 p.m., and after twenty-four hours at 8.20 a.m. Between these intervals the subjects rested or did light routine work. The average results for the control tests are recorded in table 9. They show first, that when working without breakfast, both of the subjects worked longer, with greater force, pulse rate and blood pressure the first period, than they did one hour later, indicating that they had not fully recovered from the previous hour's fatigue. Second, that both subjects worked longer, did more work, and their pulse and blood pressure increased more after the 4.20 and 8.20 p.m. periods; that is, four and two hours following the meal, than they did the first hour in the morning. This indicates that they had recovered from the fatigue of the previous work and were benefitted by the preceding meal. Third, repeating the tests the following morning at 8.20 it was observed that practically all the conditions and data were the same as they were the previous morning. Fourth, "B" did one and one-third more work, had a higher pulse and blood pressure than "A" at the beginning of work, but at 8.20 p.m. his power had lessened, although his pulse and blood pressure had not. Since at this period "A" did his best work, it happened that at this hour his power for work practically equaled that of "B."

A study of the data secured on the duration of the effect of 1.42 grain of caffeine, brings out the interesting results that now both

TABLE 9

Effect of caffeine after one to twenty-four hours

SUBJECT	CONDITION	ORDER OF WORK	DOSE	LAPSE OF TIME AFTER DOSE	TIME BEGAN WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAM-METERS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			grains	*hours*									
Ia Control	No breakfast. Began work	1			8.20 p.m.	3′ 25″	68	6,579	1,644.7	76	100	108	120
	Rested 1 hour. Began work	2		1	9.20 a.m.	2′ 57″	59	5,630	1,407.5	72	92	114	120
	Lunch at 12.30. Routine work of day. Began work	3		8	4.20 p.m.	3′ 53″	78	7,864	1,966.0	76	112	116	130
	Routine work of day till 6 p.m. Dinner at 6.30. Light reading. Began work	4		12	8.20 p.m.	4′ 30″	90	8,935	2,233.7	76	108	116	138
	Next morning 7½ hours sleep. No breakfast. Began work	5		24	8.20 a.m.	3′ 24″	68	6,576	1,644.2	76	100	108	120
Ib Control	No breakfast. Began work at	1			8.20 a.m.	4′ 05″	82	8,904	2,223.5	76	108	128	148
	Rested 1 hour. Began work at	2		1	9.20 a.m.	3′ 25″	68	6,732	1,683.0	64	92	142	152
	Routine work of day. Lunch at 12.30 p.m. Began at	3		8	4.20 p.m.	4′ 25″	88	9,634	2,408.5	82	116	132	164

Ib Control	Routine work of day until 6 p.m. Dinner 6.30 p.m. Light reading	4		12	8.20 p.m.	4′ 20″	87	9,888	2,272.0	72	109	128	162
	Eight hours sleep. Next morning no breakfast. Work	5		24	8.20 a.m.	4′ 08″	83	8,936	2,234.0	74	106	128	146
IIa	No breakfast	1			8.20 a.m.	3′ 20″	66	6,827	1,706.7	72	108	110	144
	Rested until 9. Took 1.42 grains caffeine. Began work 20 minutes later	2	1.42	½	9.20 a.m.	5′ 05″	102	10,342	2,588.5	84	124	110	144
	Routine work of day. Lunch at 12.30 p.m.	3		7	4.20 p.m.	4′ 18″	86	8,935	2,233.7	80	124	110	140
	Routine work of day until 6 p.m. Dinner, light reading. Work	4		11	8.20 p.m.	7′ 07″	142	11,291	2,822.7	72	130	110	136
	Seven and one-half hours sleep. Next morning no breakfast. Work	5		23	8.20 p.m.	4′ 02″	81	8,415	2,103.7	72	120	112	132
IIB	No breakfast. Began work at	1			8.20 a.m.	3′ 55″	78	8,629	2,157.2	76	104	128	164
	Rested till 9. Took 1.42 grains caffeine. Began work 20 minutes later	2	1.42	½	9.20 a.m.	5′ 35″	112	12,025	3,006.2	84	116	138	162
	Routine work of day. Lunch at 12.30 p.m. Work at	3		7	4.20 p.m.	5′ 20″	107	12,178	3,044.5	76	116	138	152

TABLE 9—*Continued*

SUBJECT	CONDITION	ORDER OF WORK	DOSE	LAPSE OF TIME AFTER DOSE	TIME BEGAN WORK	DURATION OF WORK	NUMBER OF CONTRACTIONS	HEIGHT LIFTED IN CENTIMETERS	WORK DONE IN KILOGRAMS	PULSE BEFORE WORK	PULSE AFTER WORK	BLOOD PRESSURE BEFORE WORK	BLOOD PRESSURE AFTER WORK
			grams	*hours*									
IIB	Routine work of day till 6 p.m. Dinner 6.30 p.m. Light reading. Work at....	4		11	8.20 p.m.	4′ 35″	92	9,180	2,295.0	80	120	140	168
	Eight hours sleep, work. Next morning no breakfast.....	5		23	8.20 a.m.	4′ 20″	67	8,976	2,244.0	76	108	128	144
IIIA	No breakfast.....				7.00 a.m.	4′ 10″	83	7,466	1,866.5	72	108	112	128
	No breakfast. Took 2.84 grains caffeine at 8 a.m.....	1	2.84	½	8.30 a.m.	4′ 38″	92	10,189	2,541.2	68	120	120	140
	Next morning, no breakfast. Work at	5		24	8.30 a.m.	4′ 13″	84	8,904	2,223.5	80	116	114	132
IIIB	No breakfast.....			½	7.00 a.m.	5′ 25″	107	11,444	2,861.0	78	106	134	148
	No breakfast. At 8.15 took 2.84 grains caffeine. Began work	1	2.84		8.45 a.m.	9′ 55″	198	22,338	5,584.5	88	120	140	168
	Next morning. No breakfast. Work at...	5		24	8.30 a.m.	7′ 30″	150	15,330	3,837.5	60	104	128	158

subjects did more work the second period than they did the first; that is, one hour after working without breakfast and twenty minutes after taking caffeine, and there was no indication of fatigue due to the previous hour's work but rather signs of stimulation, or inhibition of fatigue. Their pulse rate was much greater, but their pressure was little changed. Moreover, even after twenty-four hours, they still did more work than they did at the same hour of the previous day, and their pressure and pulse rate were no higher than at that time. As *without*, so now *with* caffeine for the periods chosen for observation "A" displayed his greatest power for work at 8.20 p.m. He did now one and one half times as much work as was possible without the drug, and what is remarkable, did at least as much work at that particular time as "B" could perform.

Concomitant with the great amount of work on "A's" part, there was an enormous acceleration of fifty-eight heart beats per minute and an increase of 26 mm. Hg in blood pressure which was a fall of 8 mm. Hg from the increase in pressure due to work without breakfast.

"B" now did his best work, as before, without eating breakfast with the 1.42 grain of caffeine at 4.20 p.m., or seven hours after taking the dose and four hours after luncheon. He did more work than at any of the other periods tested. His heart rate increased forty beats per minute, but his blood pressure increased only 14 mm. Hg which was much less and his heart beats considerably more than when working without eating breakfast.

With the stronger dose of 2.84 grains of caffeine, "A" did one and one-third as much work as was possible without the drug, but did no more than he was able to do twenty minutes after taking the weaker dose of 1.42 grain. "B" did twice as much as he did without the drug, almost twice as much as he did at his best with the weaker dose, and twice as much as "A" was able to do with the stronger dose.. "B's" pulse and blood pressure were increased, the former more so than with the weaker dose, while "A's" pulse rate fell slightly after taking the drug but was almost doubled after the work, and his blood pressure showed less rise than it did with the weaker dose.

After twenty-four hours, however, both subjects did much more work than they did at their best without eating breakfast, showing that the after effect of the strong dose not only persisted twenty-four hours, but that it still had a very powerful effect that would probably continue considerably longer. The strong dose, after twenty-four hours, caused a great depression of "B's" pulse rate far below normal

and an increased rate above normal in "A's". In both subjects the pulse was greatly accelerated by the work, indicating a heightened irritability to stimuli produced by the work at that time. Therefore the stimulating influence of the caffeine persisted at least twenty-four hours after the doses were taken. There seems also to be an optimum period when the effects were more pronounced, and a maximum dose limit beyond which the power for muscular work is lowered. This period and limit varies in different individuals.

When these investigations were undertaken, "B" complained of weakness of his eyes, and on November 3, 1912, was fitted with glasses. On June 3, 1913, during the time he had been experimenting with the larger doses of caffeine and after he had taken one of the strongest doses, the external rectus muscle of his left eye became paralysed. This may have been a weak muscle and readily affected by the drug. The paralysis of the muscle incapacitated him for further work. At about this time "A" also complained of being very irritable and feared that the caffeine was detrimental to his health. It seemed to him that he could work indefinitely without fatigue yet his muscles failed to contract and his heart beat at a tremendous rate. The experiments were therefore discontinued and the study of the after effects of caffeine, which had been planned and just begun, had to be abruptly abandoned. The indications were that there were after effects that interfered with efficiency of physical and mental activities. "B's" friends, among them two physicians, charge it to the influence of the caffeine that "B" failed in an athletic exhibition in which he took part in the spring during the time that he was conducting the caffeine experiments. Before he began the experiments he had trained himself so that he was able to hit the punching bag with his head, feet and hands alternately on its rebound. It required speed, accuracy and control of muscles, and concentration of thought. He had become an expert in this feat. But his power of concentration, accuracy and precision in his muscles had been greatly impaired so that he was unable to repeat the athletic demonstration with any credit during the time he was taking the strong doses of caffeine.

DISCUSSION

In the two subjects on which the tests were conducted, the pulse rate, blood pressure and power and duration of muscular contraction, in the trained as well as the untrained muscles, were more or less differently affected by the following conditions: the same doses of caffeine as well as the doses of caffeine per kilo body weight; no breakfast; breakfast without caffeine; and the interval following either breakfast or a dose of caffeine.

Lombard (1), by repeating his ergographic tests several times daily, quadrupled the power of his untrained flexor in twenty-two days. Food increased his power for muscular contraction, and he accomplished

more work one hour after than one and one-half hour after a meal. He found that the usual amount of coffee did not affect his power for muscular contraction. The results obtained by the athlete and the non-athlete corroborate those found by Lombard, but do not agree with his conclusions in reference to the effect of the usual amount of a cup of coffee. The difference may possibly be due to the fact that the effects of caffeine as a rule do not disappear in twenty-four hours, and therefore drinking this daily would keep the subject constantly under its influence and he could not compare its effect with those obtained with no caffeine. The athlete had seldom in his life, and the non-athlete only moderately, partaken of coffee, which facts may explain why the athlete was affected more strongly and more rapidly by the drug, than was the non-athlete or Lombard. The athlete felt generally better when working without breakfast, and both subjects did more ergometer work two and one-half hours after than one hour after eating. The pulse rate was much more accelerated in the athlete, in fact often more than double that of the non-athlete, and this may account for the greater fatigue of the athlete from work after eating breakfast.

The conclusion of previous workers that an optimum dose of caffeine increases the capacity for muscular work and inhibits the sense of fatigue, and that a larger dose decreases the power for muscular contraction was confirmed. It was also found that an optimum dose of caffeine may double the capacity for work over that produced at any time after eating breakfast; that it inhibits the sense of fatigue for many hours after the most exhaustive work with the ergometer; and that when the optimum dose, which was different per kilo weight in the two subjects was increased the working power and blood pressure were decreased, the heart rate accelerated, and with a very large dose was inhibited. Rivers and Webber's (2) results are in harmony with these. They found that 1 to 3 grains of caffeine stimulate and 4 to 6 retard the speed of typewriting, and that the relation between blood pressure and pulse rate is not constant, and that strong doses accelerate the pulse but depress the blood pressure. These results, according to Sollmann and Pilcher (3) and other investigators, are held to be due to the stimulating effect of the optimum dose on the muscle and cardiac tissue, and the depressing effect on the nervous system that controls the sense of fatigue. The large doses stimulate the muscle and cardiac tissue more, and if increased above a certain amount produce a progressive depression of these tissues. The large dose also inhibits the peripheral vasomotor mechanism, causing dilation of the blood vessels,

and at the same time stimulates the vasomotor center. These two effects neutralize each other more or less, and thus produce changes in the blood pressure that are proportional to the extent of neutralization.

Sollmann and Pilcher (3) also observed that large doses may be followed by paralytic phenomena, causing fatigue and depression of muscular contractions.

It was interesting to find that the effect of the same dose varies considerably with the interval of time after the drug is taken. In the athlete, the effect of the weak dose of 1.42 grain of caffeine gradually increased from twenty to forty-five minutes, and in the non-athlete from twenty minutes to three hours after the drug was taken—that is, this dose was only five-eighths as strong per kilo body weight for the athlete as it was for the non-athlete, but had its maximum effect in the athlete in two-eighths of the time that it did in the non-athlete. It was of shorter duration, less stimulating to muscular contraction and less effective in inhibiting fatigue; but it had a greater stimulating effect on the pulse rate of the athlete, and but little more on his blood pressure forty-five minutes after it was taken, than it did at the same interval of time on the non-athlete. Therefore, for each indivdual, different doses of caffeine may exert their optimum influence at definite intervals after the dose is administered.

When taken per kilo body weight, the effect of doses of caffeine taken at definite intervals before beginning work was different in the two subjects. Of the two doses, 0.2 grain per 9.3 kilo body weight, and 0.2 grain per 5.9 kilo body weight, and thirty minutes after the drug was taken, the weaker dose proved more stimulating to the working power of the athlete, and the stronger dose to that of the non-athlete. At the same time, the pulse rate was enormously increased in both subjects, while the increase in blood pressure was the same in the athlete as with the stronger dose, and less in the non-athlete than with the weaker dose.

It was shown throughout the experiments that the athlete was more sensitive to the effects of the caffeine than was the non-athlete, and therefore, a dose that would prove stimulating to the athlete's power of muscular contraction and heart action might prove less so for these functions in the non-athlete at that particular time.

For comparative work it therefore seems advisable to study the effects of the dose irrespective of body weight as well as of definite doses taken per kilo body weight of the subject.

Another factor that deserves consideration is that the observations ought to be made at certain periods of the day. Lombard and other investigators found that there were diurnal variations that had an influence on the power for work and recovery from fatigue. Lombard observed that his power for muscular contractions was greater from 5.30 to 6.30 p.m. than from 3.30 to 4.30 p.m., and greater at 4.30 p.m. than 11.30 a.m. Maggiora (4) held that recovery from fatigue was more rapid before 10 a.m. than at 11 a.m. These observations are strengthened by those obtained in studying the influence of caffeine and no breakfast at definite periods of the day. It was found that without eating breakfast, and also with the weak dose of caffeine, the power for muscular contractions in the athlete was greater at 4.20 p.m. than at 8.30 a.m. or 8.20 p.m. and in the non-athlete under the same conditions at 8.20 p.m. than at any of the other periods of the day tested. In both subjects, moreover, the effects of the caffeine continued at least twenty-four hours after the drug was taken. In Hollingsworth's (5) subject the stimulating effect of caffeine was noticeable even after three days. Kraeplin also observed that strong doses of caffeine retarded the transformation of intellectual conceptions into actual movements. These after effects of caffeine are explained by Cushny (6) as the results of stimulation of the central nervous system that is associated with psychical activity. After a strong dose of the drug connected thought is rendered more difficult and impressions follow each other so rapidly that attention is destroyed, and it requires more effort to limit it to a single object. These views would explain why the athlete, during the time he was taking strong doses of caffeine, was unable to perform the athletic feats in which he had excelled before conducting these experiments.

SUMMARY

The general results of the experiments recorded in this paper are as follows:

The working power of the untrained flexor muscles in a trained athlete may be increased at least three and one-half times, and the same muscle in a non-athlete three times in one month of daily training. From the first the athlete did one and one-half, in less time, and later at the same stage of training, twice as much work as was done by the same muscles in the non-athlete.

The lack of breakfast had at first a slightly less favorable effect upon the amount of work done, although the athlete always felt less fatigued

working without breakfast than when working one hour after the meal. Both subjects were able to do more work on the ergograph when the muscles were in training after eating breakfast, and more one hour than one and one-half hour following the meal. After taking 1.42 grain of caffeine, both subjects did more than twice as much work than they were able to do after eating breakfast. The after effect, however, was a heightened degree of irritability especially noticeable in the athlete. The ergographic work had practically no effect on blood pressure and only a slight effect, if any, on the pulse rate, when working either without or after eating breakfast. The normal pulse rate was practically the same, but the normal blood pressure was higher at all times in the athlete than in the non-athlete.

After both subjects had had equal preliminary training for one month of the arm and trunk muscles on the ergometer, the athlete did more than twice the work done by the non-athlete in one and one-half the time without, and more than one and one-half as much work, after eating breakfast. The efficiency of both subjects grew in proportion as the interval between the meal and beginning of the work increased from one to two and one-half hours. The non-athlete did one-half and the athlete one-third more work two and one-half hours after than they were able to do one hour after the meal.

The increase above their normal blood pressure after working either with or without breakfast was the same for both subjects, notwithstanding that the athlete did more work. But under the same conditions the pulse rate in the athlete was practically double that in the non-athlete. The increase in heart rate was least in both subjects when working two and one-half hours after eating breakfast, that is, the time when the greatest amount of work was accomplished.

A weak dose of 1.42 grain of caffeine, without work or breakfast, gradually increased the pulse rate during the first hour, but in the non-athlete as a rule only after a slight initial fall. In both subjects the pulse returned to the normal rate within three hours. With the larger dose, 2.24 grains, under the same conditions, the increase in pulse appeared more promptly, but in thirty minutes was depressed below normal in the non-athlete, and accelerated above the normal rate in the athlete. The blood pressure rose above the normal level in one hour and frequently had not returned to the level in three hours after taking either of the doses of caffeine.

The effects of caffeine taken at different intervals before work, varied with the dose and the individual. In the athlete the maximum influ-

ence of a dose of 1.42 grain was manifested in three-quarters of an hour, and in the non-athlete three hours after the dose was taken. The athlete did but little more work forty-five minutes after than he did twenty minutes after taking the drug. But the non-athlete did two and one-half times as much work three hours after as he did twenty minutes after taking the dose.

Power and endurance for work, and cardiac activity and increase in blood pressure do not keep pace with increase of dosage. The maximum power for work in both subjects was attained with the dose of 2.24 grains of caffeine. With this dose both subjects did two and a half times as much work as they were able to do one hour after eating breakfast. In the athlete with this optimum or with the weaker dose of caffeine, the blood pressure was no greater than after the maximum work done either with or without breakfast, and the heart rate was only slightly more accelerated. In the non-athlete the pulse rate was increased almost three times as much, but the blood pressure was no higher than it was after the maximum work following the meal. A stronger dose of 3.58 grains depressed the muscular power for work in both men, but very markedly so, as well as the blood pressure and pulse rate in the non-athlete. In the athlete the blood pressure was no different, but the heart rate was less after the work following the weaker dose. When the dose was taken in proportion to the body weight, e.g., 0.2 grain of caffeine per 9.3 kilo body weight, or a stronger dose of 0.2 grain per 5.9 kilo weight, the results presented another viewpoint to those obtained when the dose was taken irrespective of body weight. The facts showed that of these two doses thirty minutes before beginning work, the weaker dose and not the stronger stimulated the working power in the athlete most. But in the non-athlete the reverse was the case. With the stronger dose the athlete did one-fourth less and the non-athlete one-fourth more work than with the weaker dose. At the same time the pulse rate was enormously increased in the non-athlete and less so in the athlete who did double the work done by the non-athlete. On the other hand, the blood pressure fell slightly in the non-athlete, and fell also or remained unaltered in the athlete after work and after taking the stronger dose. Therefore, for each subject there was a definite optimum dose which, when increased, proved depressing for muscular work, blood pressure and pulse rate.

One hour's rest did not remove the sense of fatigue produced by the ergometer work, but when caffeine was taken the fatigue of the previous

hour's work was inhibited and both subjects did more work then, and even twenty-four hours after taking caffeine, than they did before taking the drug. With the same dose of caffeine and also without eating breakfast, the power for muscular work in the athlete was greater at 4.20 p.m. and in the non-athlete at 8.20 p.m. than at 8.20 a.m. That is, the athlete did his best work eight hours after taking the caffeine and four hours after luncheon, and the non-athlete did his best work twelve hours after taking the dose, and two hours after dinner. At these respective periods, the pulse rate and blood pressure increased greatly in the non-athlete, and the pulse but not the pressure in the athlete. The after effect of the larger dose was a heightened condition of irritability that persisted many hours after the drug was taken. The power and endurance for work were increased, and the cardiac activity greatly affected, but the blood pressure less so than with the stronger dose. It was not possible to state how long the after effect would endure, because the experiments were suddenly interrupted by the paralysis of the rectus muscle of the left eye in the athlete, and the nervous condition of the non-athlete.

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THE EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

IV. Effect of Massive Doses on the Outflow from Muscle

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That intravenous injections of adrenin in all dosages cause dilatation of the vessels of the muscles of the limb was recently reported from this laboratory (1). The largest dosage used in that investigation was 5 cc. of a 1–25,000 solution. Inasmuch as such dosages as these are probably many times greater than any single discharge from the normal adrenal glands, dosages of higher concentration were not studied. Also we saw no reason to believe that dosages of higher concentrations than those used would alter the reaction.

Recently Cannon and Gruber (2) published some muscle contraction graphs of animals under the influence of large doses of adrenin. Following the injections the contractions were diminished in height—a fact which suggests that vasoconstriction in the muscle might be occurring. Indeed the authors postulate this condition. Later we were informed by Dr. W. J. Meek that he had observed vasoconstriction in the muscles due to very large doses of adrenin. Gruber (3) reported that in perfused muscles, with the nerve supply cut, adrenin in *small doses* produces vasoconstriction. Dilatation to be sure could not be expected with the vessels in a state of extreme relaxation due to the loss of the tonic effect of the constrictor nerves when the vessels are supposedly dilated to their limit. Cannon and Lyman (4) have observed that after the blood pressure has reached a certain low level, intravenous injections of adrenin will no longer exert a depressor effect. In view of the foregoing observations it was decided that further experimentation on the effect of adrenin on the circulation in the muscles was desirable by way of supplementing our former communication.

METHOD

The same methods of investigation were used as were described in the first paper of the series (1). As before, dogs were used for experimentation. Volume curves were not recorded. The observations were all made on venous outflow.

RESULTS

In investigating this phase of the problem, small doses were used at first and the amount gradually increased. When the dosage reached

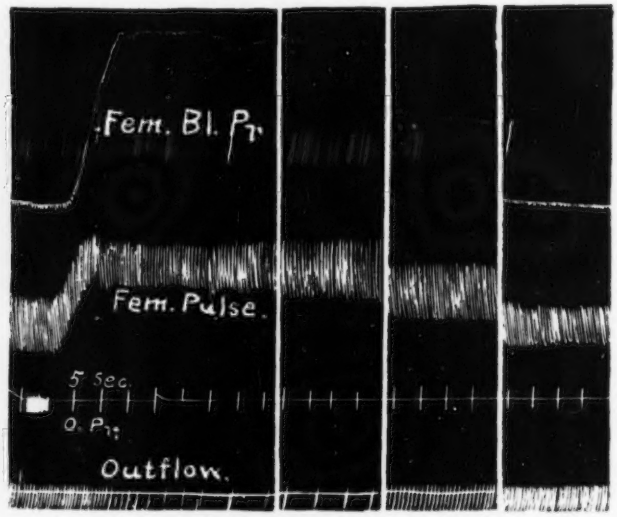

Fig. 1. Successive segments of graph showing effect of massive dose of adrenin on blood pressure, femoral pulse and venous outflow from muscle. Interval omitted in each case two minutes. Dose 1 cc. 1: 1000 "Adrenalin." Dog; weight, 11 kilos. No reduction.

1 to 2 cc. of a 1–1000 solution the reaction of pure dilatation, such as was previously reported, changes as is shown in the accompanying figure. There is a short preliminary increase in the outflow following the injections which lasts about ten seconds and occurs during the rise in blood pressure. This is probably due to the vasoconstriction forcing the blood out of the vascular spaces into the veins. At the time,

or shortly after the blood pressure reaches its crest, an active vasoconstriction takes place in the muscle circulation. This is evidenced by a diminished outflow which lasts, when the injections do not produce a too long maintained blood pressure reaction, until some time after the blood pressure has again returned to normal. In the long maintained blood pressure reactions the diminished outflow begins its return to normal several seconds after the blood pressure commences to drop. The outflow, after returning to normal, invariably showed a tendency to "over recovery" by a second increase in the rate of outflow. This secondary increase might be due to the release of blood dammed back by constriction in the veins of the muscles as was observed in the mesenteric circulation by Henderson (5) from mechanical stimulation of the intestines. It was observed, however, that there was no recovery from this secondary dilatation and also that the tendency to it was more marked toward the end of an experiment. This "over recovery" then is due most probably to a fatigue of the vascular musculature.

That the vasoconstriction was not taking place in fatigued or overdilated vessels was proven by the facts that the blood pressure was maintained, that the vasoconstriction would take place early in an experiment and that small dosages of adrenin would produce pure dilatations at any time during the experiment.

The threshold for the vasoconstrictor effect was found to vary both in the same dog and in different dogs. It was found to be slightly lower with the prolongation of an experiment and with an increase in the concentration of the injection. It was observed that 5 cc. of 1–5000 solution might produce in a given animal pure local dilatation without a marked general blood pressure reaction, whereas 1 cc. of a 1–1000 solution would produce the vasoconstriction reaction as described. The quantity of adrenin in both cases was the same. These results are probably due to the fact that the concentrated solutions reach the tissues still in more concentrated form. The solutions of lower concentration, since they are greater in volume, amount in fact to a short lasting infusion.

DISCUSSION

These results hardly have a bearing on our main thesis which is concerned with suprarenal physiology. The high dosages necessary to produce vasoconstriction in the muscles are not physiologic. They are rather to be considered as pathologic. A study of the blood pres-

sure curves, induced by these massive doses, gives the impression of a tetanus. The fact that a maintained "over recovery" occurs suggests a toxic effect which has injured some part of the vasomotor apparatus. It is a well known fact that adrenin in very large doses does produce toxic effects. It is extremely unlikely that the normal adrenal glands could at any one time pour such quantities as these into the circulation. The normal discharge, judging by all data now available, is similar to an experimental infusion of low concentration.

SUMMARY

1. Adrenin in massive dosages produces a diminished circulation in the skeletal muscles.

2. There is a preliminary increase in the outflow due supposedly to the blood present in the vascular spaces being forced into the veins by the vasoconstriction.

3. There is a maintained secondary dilatation due probably to a fatigue of the vascular musculature.

4. The vasoconstrictor threshold was found to be lowered by an increase in the concentration of the adrenin and by the repetition of injections.

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A correction. In the first paper of this series (1) the statement was made that hydremic plethora serves to convert a vasoconstrictor effect of adrenin in a limb to a vasodilator effect. Subsequent investigation has failed to corroborate our earlier observations on this point.

EFFECTS OF ADRENIN ON THE DISTRIBUTION OF THE BLOOD

V. Volume Changes and Venous Discharge in the Intestine

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Although a number of investigators have studied the effects of adrenin on the activity of the intestines relatively little attention, apparently, has been given to the effects on vascular conditions. Oliver and Schaefer (1) made no direct determinations but concluded from inspection that vasoconstriction in the gut wall follows the administration of adrenin. The generalization was offered that this substance, or, more specifically, suprarenal extracts, cause vasoconstriction throughout the splanchnic area. Elliott (2) has reported a single instance in which he painted adrenin on the wall of the intestines of a fowl and also gave an intravenous injection. The result as determined by inspection was an intense vasoconstriction.

Froelich (3) in a brief Centralblatt article has reported that both *d*- and *l*-suprarenin as well as "adrenalin" caused long lasting contraction of a segment of gut enclosed in a plethysmograph. He used both cats and dogs. Few details as to the experiments were given. Vincent (4) states that sometimes the intestine expands under the influence of adrenin and publishes a plethysmograph curve that shows a slight degree of expansion that might be interpreted as passive.

Brodie and Dixon (5) noted that the addition of adrenin to a perfusate materially decreased the flow through the vessels of the isolated intestines.

Ogawa (6) included the gut in a series of perfusion studies on the vasomotor effects of adrenin. Rabbits, cats and dogs were utilized as experimental animals. It was reported that *l*-adrenin in greater concentration than 1:5,000,000 caused a clean-cut constriction in the intestinal vessels, as was shown by a decreased venous outflow. With the higher concentrations occasionally a secondary dilatation was ob-

served. In higher dilutions, e.g., 1: 50,000,000, the effect was primary dilatation. It was found that *d*-adrenin had a similar effect but larger doses were required.

Technique. In our investigations both plethysmograph studies and determinations of the outflow from the opened intestinal veins have been made. Ether anesthesia was used in all cases. The adrenin solutions were made with Parke, Davis "Adrenalin" in distilled water. The technique employed has been described in sufficient detail in the first two papers of this series (7). It need only be added that in the plethysmograph work segments of small intestine about 20 cm. in length were used. These were coiled or folded into the box with due care, of course, to prevent occlusion of the blood vessels. The prompt volume changes and frequently the appearance in the tracings of vascular pulsations showed that success in this respect had been achieved. In all some three hundred and twenty-five determinations were made on forty-five dogs. In the infusion experiments the duration varied from one-half to nine minutes.

Results. The results of the experiments are summarized in the accompanying table. In many instances a brief inconsequential preliminary dilatation was observed but as this is a common feature in all the organs studied and probably is merely a passive effect, it is ignored in the table and subsequent discussion. The most prominent features in the results as a whole were augmentation of the gut volume and of the discharge from the opened veins. Often, however, the dilatation phase was preceded by a more or less pronounced contraction and in some instances contractions only were obtained. The only possible combination of these two conditions that was not encountered was dilatation followed by contraction. In case of the venous outflow the results were somewhat more consistent an augmentation always having occurred but this was not infrequently preceded by a decrease during the first part of the reaction.

There was no very definite correlation between the dosage and the reaction in the gut. In some animals smaller quantities caused contraction which was succeeded by dilatation when the amount of the adrenin was increased. In other animals, however, the reaction remained constant except as to degree whatever quantity was injected. The doses varied in the injection experiments from 0.25 to 8 cc. of 1: 100,000 solution. The effects of massive doses were not investigated but the upper range actually employed very materially transcended physiological limits so far as these can be determined from data now

available. The data in the accompanying table are reduced roughly to a quantitative basis. Since the dogs used were not greatly dissimilar in size (averaging about 12 to 13 kilos), while the variations in reactions among various animals were much greater, nothing would

Effects of adrenin in the small intestine

	NORMAL		AFTER ECK FISTULA	
	Number of cases	Average dose	Number of cases	Average dose
		mgm.		*mgm.*
1. On volume				
a. Injections				
Pure contraction	9	0.013	0	
Pure dilatation	52	0.021	7	0.034
Contraction followed by dilatation	90	0.023	52	0.026
Dilatation followed by contraction	0		0	
		mgm. per minute		*mgm. per minute*
b. Infusions				
Pure contractions	2	0.011	0	
Pure dilatations	15	0.030*	8	0.043
Contraction followed by dilatation	14	0.036	11	0.046
Dilatation followed by contraction	0		0	
		dose in mgm.		*dose in mgm.*
2. On venous outflow				
a. Injections				
Pure augmentation	9	0.020	0	
Diminution followed by augmentation	31	0.025	2	0.020
		mgm. per minute		*mgm. per minute*
b. Infusions				
Pure augmentation	4	0.030*	0	
Diminution followed by augmentation	6	0.030	2	0.050

* Excluding one case of very low irritability.

be gained by expressing the dosages more definitely as milligrams per kilo. The average of all doses giving each effect is stated. Such averages are of restricted value but so far as they go they indicate in gen-

eral a tendency toward constriction with smaller doses and a dilatation with larger. It may be reiterated, however, that in individual cases this statement often does not hold. As previously mentioned, Ogawa (6) obtained dilatations with smaller rather than larger doses.

Unlike the other organs studied, the gut frequently gave reactions with injections different from those obtained with infusions. In various instances with injections a definite contraction phase was noted whereas in the same animals infusions gave pure dilatations. These dilatations were observed both with dosages which caused little change in blood pressure and with those causing a sustained rise. This is a feature worthy of note in consideration of the adaptive significance of the suprarenal glands. If, as there is considerable reason to believe, adrenin discharge plays a significant part in integrating the body for muscular exertion, dilatation of the intestinal vessels would seem to be a detriment. It is quite possible, however, that the exposure of the splanchnic organs and the incidental trauma may be a determining factor in the reactions noted and thus account for the discrepancy. The dilatation is probably correlated with the well known fact that adrenin under ordinary experimental conditions causes flaccidity of the gut. This in itself would supposedly have a material tendency to augment the caliber of the intestinal vessels and might even mask a primary vasoconstrictor effect. This is borne out by the fact that in two animals small doses produced sustained enterocontraction.

The threshhold of reaction in the gut was found to be in general about the same as that of blood pressure. The enteric reaction, however, usually persisted for some time after blood pressure returned to normal. This lag in the volume reactions has been observed very generally in all the organs studied. Often in infusion experiments the blood pressure is briefly shifted but returns essentially to normal whereas the volume change persists throughout the time the drug is being administered. This fact would seem to prove that there is a series of surprisingly delicate and efficient reflex interconnections among the various organs whereby a change in the vascular conditions of one is compensated by a change of an opposite character in another so that the general blood pressure and hence the circulation in the brain and various "neutral" organs remains practically undisturbed.

In case of both injections and infusions there was frequently observed an after dilatation. In cases in which the primary effect was enterodilatation this appeared merely to a prolongation of the reaction but where the reaction proper was enterocontraction it amounted to a dis-

tinct over-recovery. This after-effect usually persisted two or three minutes.

In such cases as those shown in figures 1 and 3 in which enterocontraction occurred, the gut reaction frequently synchronized with a vascular hypertension and supposedly played a considerable rôle in its production. In many cases, however, the gut dilated while the general blood pressure either remained essentially unchanged or even, as in figure 2, considerably increased. In some instances when enterocontraction occurred it outlasted by a considerable period the hypertension. These facts show that although vasoconstriction in the intestines may play a considerable part in augmenting systemic blood pressure there are other structures which have enough greater influence even to cause hypertension synchronous with enterodilatation. These other organs are probably the liver, kidneys, spleen and skin.

In view of the fact that the blood from the intestines has to pass through the liver, an organ which contracts under the influence of adrenin, (8) the question arises: To what extent are volume reactions in the intestine dependent upon back pressure from the liver? To answer the question several dogs were tested before and after the production of Eck fistulae. These were made by the well known method of stitching together the portal vein and the vena cava so as to hold in hermetically tight apposition an elipsoid area of their external walls. Then by traction and sawing with a cutting suture previously placed the walls were ruptured so as to establish a free connection between the veins. When the portal vein was ligated between the liver and the fistula one could make certain not only that the opening was patent, but also, if no evidence of back pressure developed, that the fistula was of adequate size. Having performed all the operation up to the last stage the adrenin reactions were determined. Then the fistula was quickly made and the portal vein ligated and the adrenin administration repeated. In one case the result of shunting the portal blood directly into the vena cava was to convert an enterodilatation to an enterocontraction but in the other instances the reactions were essentially unchanged. (See tabulated results.)

The effect of adrenin on the outflow from an open intestinal vein was purely or predominantly an augmentation, amounting at times to 300 per cent (fig. 3). In cases where the volume reaction was dilatation this result would of course be the expected one but it was observed consistently throughout the series irrespective of whether the gut dilated or contracted or whether arterial pressure was raised

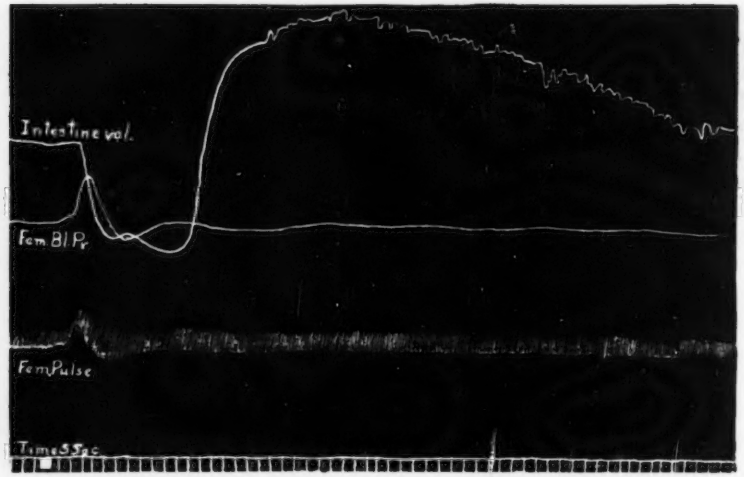

Fig. 1. Graph showing effects of adrenin injection on gut volume, femoral blood pressure and femoral pulse. Dose, 1 cc. 1: 100,000. Dog weight, 16 kilos. Time, five seconds. Reduced to one-half.

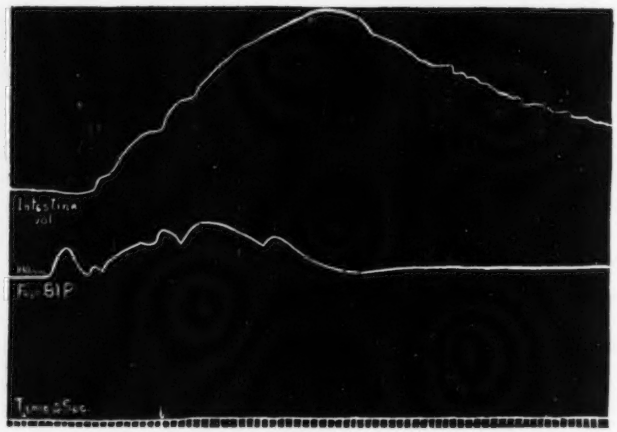

Fig. 2. Graph showing effects of adrenin infusion on gut volume and femoral blood pressure. Adrenin 15 cc. 1: 200,000 in ninety seconds, from *a* to *b*. Dog weight, 16 kilos. Time, five seconds. Reduced to one-third.

or lowered. Since any vein large enough to cannulate is connected through anastomoses fairly directly with the portal vein the rate of outflow would depend rather more, probably, upon portal pressure than directly upon capillary changes in the gut wall. Since the outflow was consistently augmented by adrenin, one would accordingly postulate

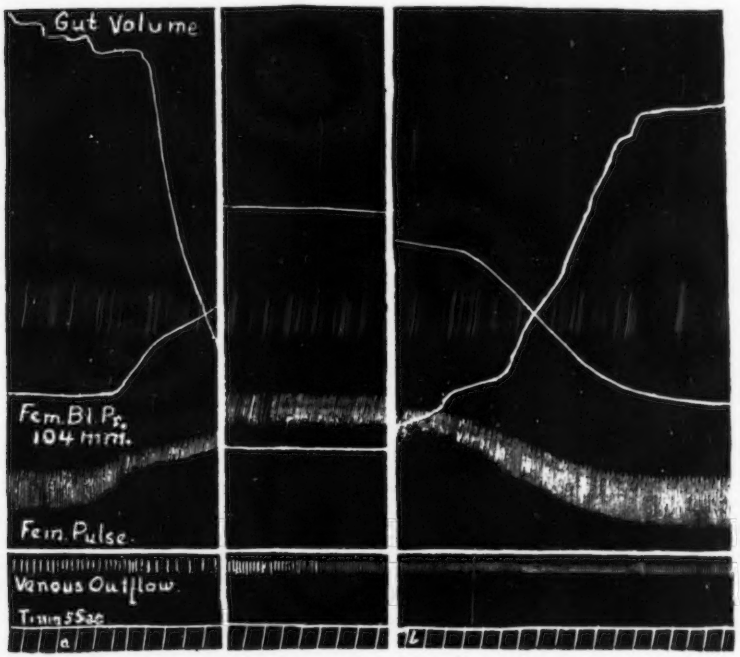

Fig. 3. Graph showing effects of adrenin infusion on gut volume, femoral blood pressure, femoral pulse and venous outflow. Twenty-two and one-half cubic centimeters adrenin, 1: 200,000 in two hundred and twenty-five seconds, from *a* to *b*. Dog weight, 16 kilos. Time, five seconds. Ninety seconds omitted between each segment of the graph. No reduction.

an increase in portal pressure with all effective doses. But that this is not an essential feature in the explanation is shown by the fact that an exactly similar reaction occurs in dogs with Eck fistulae. According to the observations of Capps and Mathews (9) adrenin in the quantities used in these experiments produces little or no effect upon the

pressure in the vena cava, hence the augmented outflow from the intestinal veins is not to be ascribed to back pressure from the large veins. The only explanation that has occurred to us is that the adrenin may produce sufficient contraction in the proximal mesenteric veins to cause considerable back pressure such as Henderson (10) has observed in surgical shock. Whether this actually is the cause was not determined.

SUMMARY

1. The effects of adrenin in physiologic doses were investigated as regards gut volume and venous outflow in anesthetized dogs.

2. There was no definite correlation between dosage and volume changes but dilatation predominated, particularly with larger doses.

3. Often the dilatation was preceded by contraction. In some instances contraction alone occurred.

4. Infusions frequently gave only enterodilatation in animals in which injections gave a preliminary contraction.

5. The thresholds for changes in blood pressure and gut volume were approximately the same.

6. In some cases enterocontraction coincided with vascular hypertension but there was no constant relation between blood pressure and gut volume changes.

7. The volume changes usually persisted after blood pressure returned to normal, indicating reflex compensatory adjustments among various organs.

8. An after dilatation of the gut was frequently noted when the primary adrenin effect had worn off.

9. In most cases Eck fistulae made no difference in the gut reactions hence the liver played no essential part.

10. The outflow from a small cannulated gut vein was augmented by adrenin in all effective doses irrespective of changes of blood pressure or gut volume. This augmentation was not due to back pressure from the portal vein or vena cava. In most cases the augmentation was preceded by brief diminution in the outflow.

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THE VASCULARITY OF THE ADRENAL BODIES

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Our knowledge regarding the innervation of the adrenal bodies is based chiefly upon Biedl's (1) determinations of the flow from the suprarenal vein during stimulation of the greater splanchnic nerve. The procedure practiced in these experiments was as follows: Having excluded the kidneys from the circulation, the inferior vena cava was ligated distally to the entrance of the renal veins. A loose ligature was then placed around this blood-vessel centrally to the orifices of the suprarenal veins. By tightening this ligature the blood of the suprarenal veins could be collected in this pouch and could be directed at any moment through a cannula into a bottle containing a solution of magnesium sulphate. The quantity of fluid displaced from the bottle was measured with the aid of an ordinary drop recorder.

In his experiments proving that the activity of the adrenal glands is controlled by secretory fibers, Dreyer (2) collected the blood from a cannula which was inserted in the left suprarenal vein distally to the gland. Stewart (3) has recently employed a method very similar to that of Biedl with this modification, however, that one of the iliac veins was tied near the cava and the other close to its point of origin. In this way it was possible to place the latter vertical, and to permit it to serve, so to speak, as the neck of a measuring flask, the body of which was formed by the trunk of the inferior cava. Having first emptied this pocket, the moment could easily be determined when the blood from the suprarenal vein first entered it and again when it reached the proximal end of the iliac vein. The quantity of blood required to fill this pouch could easily be measured by simply emptying its contents into a graduated cylinder. No statements, however, are made regarding the volume of the bloodflow in any of the papers cited.

The present experiments were originally undertaken with the intention of obtaining a more exact proof of the existence of vasomotor

fibers in the adrenal bodies than has been presented by Biedl. Certain technical difficulties, however, have prevented us from carrying them to completion. In the succeeding pages we shall confine ourselves, therefore, to a brief discussion of certain data pertaining to the vascularity of the suprarenal gland.

The experiments were performed upon five large dogs during ether narcosis. Having opened the abdomen by a median and left transverse incision, a loose ligature was placed upon the left suprarenal vein centrally to the gland. The left greater splanchnic nerve was then isolated directly below the diaphragm and placed in shielded electrodes. The suprarenal vein having been ligated at a distance of about 1 cm. distally to the gland, a stromuhr[1] was then inserted in this blood-vessel centrally to the ligature and in the immediate vicinity of the gland. Thus, by quickly changing the clip from the distal end of the suprarenal vein to its central end at the site of the loose ligature, the blood could be diverted at any moment into the stromuhr without interrupting the circulation. From the stromuhr the blood was returned to the inferior cava by way of the left renal vein. To accomplish this end it is not always necessary to ligate the renal blood-vessels because in many cases the renal vein arises from two large radicles, only one of which need be closed for the insertion of the distal cannula of the stromuhr. At the completion of the experiment the clip was again placed upon the suprarenal vein distally to the gland and the ligature unloosened on its central side. The blood then followed its usual course into the inferior cava. In all these experiments the general arterial pressure was recorded by a mercurial manometer which was connected with the left femoral artery and the venous pressure by a membrane manometer which communicated with the central cannula of the stromuhr.

The results of these experiments are compiled in tables 1 and 2. It should be mentioned, however, that only those periods of the stromuhr have been included in this calculation during which no special experimental procedure has been resorted to. It will be seen that the weight of these dogs varied between 15 and 21 kilos and that the weight of the left adrenal body varied between 0.85 and 2.60 grams. The blood-flow fluctuated between 0.069 and 0.217 cc. in a second, the pressure between 90.5 and 122.5 mm. Hg. Thus, a gland weighing 1.72 grams receives on the average 0.142 cc. of blood in a second or

[1] We have made use of the recording stromuhr described by Burton-Opitz, Pflüger's Arch., 1908, cxxi, 150.

TABLE 1

The blood supply of the suprarenal gland

NUMBER OF EXPERIMENT	WEIGHT OF		DURATION OF PHASE	TOTAL QUANTITY OF BLOOD	QUANTITY PER SECOND	BLOOD PRESSURE IN MM. HG	
	Dog	Gland				Arterial	Venous
	kg.	*grams*	*min.*	*cc.*	*cc.*		
1	20	1.38	4.0	15.2	0.063	112.0	8.5
			3.7	16.0	0.071		
			3.5	15.4	0.073		
			3.5	15.0	0.070		
Average					0.069	112.0	8.0
2	21	1.59	2.2	18.0	0.133	114.0	10.0
			2.2	17.0	0.129		
			2.5	17.6	0.116		
			2.5	18.0	0.120		
Average					0.124	114.0	10.0
3	18	2.60	1.6	18.2	0.190	110.0	10.0
			1.8	18.0	0.167		
			1.8	18.0	0.167		
			1.4	18.5	0.220		
			1.5	18.2	0.201		
			1.8	18.5	0.166		
Average					0.185	110.0	10.0
4	19	2.21	1.5	18.2	0.201	122.5	11.0
			1.2	17.0	0.236		
			1.2	18.0	0.250		
			1.2	18.0	0.250		
			1.5	18.0	0.200		
			1.6	18.5	0.193		
			1.6	18.5	0.193		
Average					0.217	122.5	11.0
5	15	0.85	2.7	18.5	0.114	90.5	8.5
			2.5	18.0	0.120		
			2.6	18.0	0.115		
Average					0.116	90.5	8.5

TABLE 2

Average values of flow and pressure

NUMBER OF EXPERIMENT	WEIGHT OF		BLOOD FLOW PER SECOND	BLOOD PRESSURE IN MM. HG	
	Dog	Gland		Arterial	Venous
	kg.	*grams*	*cc.*		
1	20	1.38	0.069	112.0	8.0
2	21	1.59	0.124	114.0	10.0
3	18	2.60	0.185	110.0	10.0
4	19	2.21	0.217	122.5	11.0
5	15	0.85	0.116	90.5	8.5
Average.....	18.6	1.72	0.142	109.8	9.5

8.5 cc. in a minute. At a pressure of about 100 mm. Hg, 100 grams of adrenal tissue are supplied with about 490 cc. of blood in a minute.

If this value is now compared with the succeeding values of the blood-flow through other organs (4) it will be evident that the adrenal gland is a very vascular organ; its blood-supply being exceeded only by that of the thyroid body.

	cc. in a minute
Post. extremity	5.0
Skeletal muscle	12.0
Head	20.0
Stomach	21.0
Liver (art.)	25.0
Portal organs, comb.	30.0
Intestine	31.0
Spleen	58.0
Liver (ven.)	59.0
Liver (total)	84.0
Brain	136.0
Kidney	150.0
Suprarenal	490.0
Thyroid	560.0

In this connection it is interesting to note that Neuman (5) has found an oxygen-consumption of 0.045 cc. per gram of substance and per minute. These tests were made upon the suprarenal of the cat, the Barcroft-Roberts differential blood-gas apparatus being used. This value indicates a flow of about 6 cc. per gram per minute.[2]

[2] The current number of the Proc. of the Soc. for Exper. Biol. and Med. which has been issued since the preparation of this manuscript, contains a preliminary notice by Stewart and Rogoff in which it is stated that the caval segment fills at the rate of about 8 cc. per minute, in a dog weighing 10 kilos.

By employing the method previously described, Biedl has found that the number of drops escaping from the reservoir may be considerably increased by stimulation of the splanchnic nerve. This acceleration, however, does not set in immediately, but about ten seconds after the beginning of the stimulation and lasts for some time after its close. Moreover, while it pursues a course which is practically parallel to the rise in blood-pressure, the fact remains that it outlasts the stimulus as well as the increase in pressure and this seems to show that it is dependent upon an active enlargement of the suprarenal blood-vessels.

These alterations in the blood-supply of this gland we have succeeded in registering a number of times, but as these curves present only quantitative and not qualitative differences, it may suffice to illustrate them by a single example taken from experiment 3. The second and third phases of this experiment are reproduced in the accompanying figure. The blood-flow (*St*) showed in this case the normal value of 0.155 cc. in a second. The arterial pressure (*A*) amounted to 100.2 mm. Hg and the venous pressure (*V*) to 9.8 mm. Hg. Shortly after the beginning of the excitation of the greater splanchnic nerve at *S*, the blood-flow increased gradually until it assumed its maximal value of 0.244 cc. in a second at about point *T*. The latter coincides with the cessation of the stimulation and also with the maximal rise in the arterial pressure. Subsequent to *T* the arterial pressure decreases gradually and assumes its normal level early in the course of the third phase of the stromuhr, about fifty seconds after the cessation of the stimulation. The venous pressure exhibits a slight rise during the period of the increased flow.

The blood-flow pursues a very similar course. It is true, however, that the flow does not always return to normal with the pressure, but remains augmented for some time after the stimulation. In the present case, for example, the normal minute-volume for 100 grams of suprarenal substance amounts to 260 cc., while the minute-volume during the stimulation measures 560 cc. It appears, therefore, that the excitation of the splanchnic nerve has resulted in an increase of flow of 200 cc. in a minute for 100 grams of substance. This fact in itself, however, does not seem to us to justify the deduction that the suprarenal blood-vessels are equipped with a dilator mechanism, because the dissociation between the flow and the pressure here noted, could also be caused by the rather slow return to normal of passively distended blood-vessels. It also seems singular that the excitation of

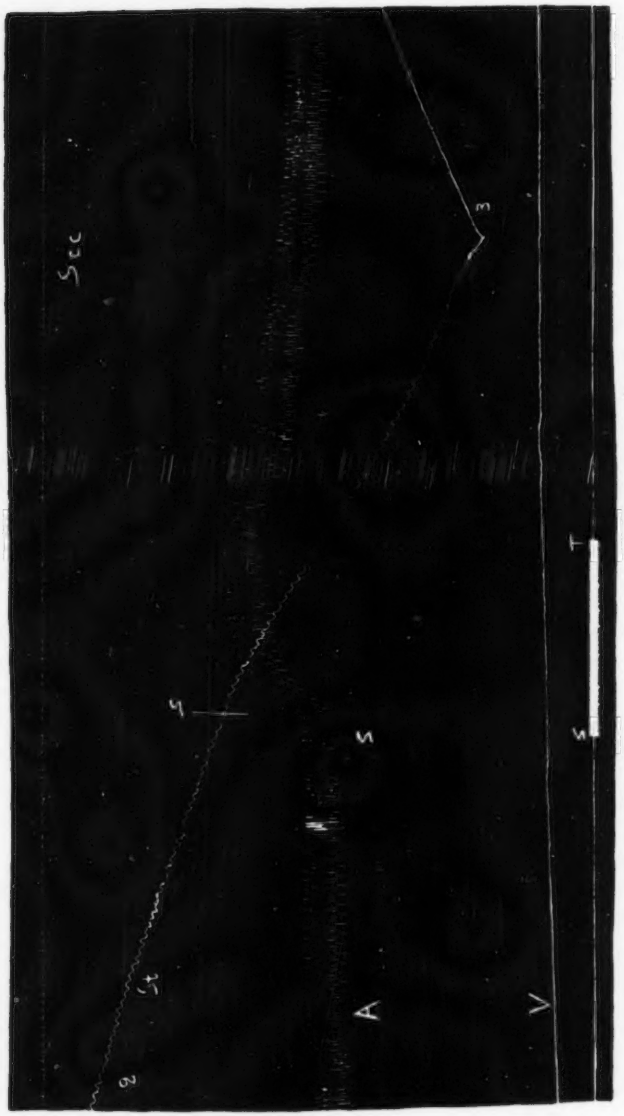

Fig. 1. The blood flow through the suprarenal gland during stimulation of the greater splanchnic nerve (15 cm., twenty seconds).

the splanchnic nerve with currents of medium strength and high frequency should dilate the blood-vessels of the suprarenal gland and constrict those of the other organs innervated by this nerve.

The possibility that the splanchnic rise in blood-pressure is the essential factor in the increased blood-supply of the suprarenal gland we have attempted to test by the simultaneous stimulation of the distal and central ends of the splanchnic nerve, the former with a current of ordinary strength and frequency and the latter with a current of low frequency. This procedure necessitated the division of the left thoracic sympathetic nerve a short distance above the diaphragm and the application of two shielded electrodes. The inductoriums were then adjusted in such a way that the fall in blood-pressure obtained in consequence of the excitation of the central end, was about balanced by the rise resulting from the stimulation of the distal end.

In this way, we succeeded in retaining the blood-pressure practically at its normal level, because the rise in pressure resulting from the constriction of the blood-vessels in the different splanchnic organs, was counterbalanced by the vasodilatation produced elsewhere. We have, however, not been able to satisfy ourselves that under this condition the blood-flow through the suprarenal gland is markedly increased. But naturally, this result cannot justly be regarded as proving the absence of vasodilator fibers, because the stimulation of the central end of the splanchnic nerve may have destroyed this effect reflexly through possible nervous connections between the suprarenal gland and the solar ganglia. If the central end of the thoracic nerve alone was stimulated, the resulting fall in blood-pressure was invariably associated with a diminution in the blood supply of this organ.

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THE INFLUENCE OF SECRETIN ON THE NUMBER OF ERYTHROCYTES IN THE CIRCULATING BLOOD

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In 1895 Dolinski (1) showed that acids brought into contact with the mucous membrane of the duodenum set up a secretion of pancreatic juice. Pawlow and his co-workers (2) further decided that the acid acts reflexly through a nerve center. Later Popielski (3), working under the direction of Pawlow, showed that if acids are introduced into the duodenum the pancreatic secretion appears after resection of both vagus and splanchnic nerves, after extirpation of the solar plexus and even after destruction of the spinal cord. He concluded that the secretion arose from a peripheral reflex through scattered ganglia of the pancreas, situated mostly near the duodenum. The same results and conclusions were reached by Wertheimer and Lepage (4). At this point Bayliss and Starling (5) demonstrated the true explanation of the phenomenon. They showed that the acid acts on a substance in the duodenal mucous membrane, prosecretin, and changes it into another substance, secretin. This is carried by the blood and activates the pancreatic cells.

Bayliss and Starling (5) also showed that secretin increases the secretion of bile. We have confirmed this by noting the rate of flow of bile incidentally in the course of other experiments. Sir Edward A. Schäfer (6) states that secretin increases the flow of bile and of succus entericus but to a less extent than it affects the flow of pancreatic juice. He also states that intravenous injection of duodenal extract (evidently secretin from the context) has been shown by Cow (7) to cause the appearance of the pituitary autacoids in the cerebro-spinal fluid.

Beveridge and Williams (8) in their very ingenious exposition of what they call the proteomorphic theory of immunity claim to have the records of over two hundred cases of diabetes and exophthalmic goiter in which the number of red corpuscles per cubic millimeter of

blood was increased by the administration of secretin. Their theory of the production of immunity depends greatly on the power to hydrolyze proteins which they attribute to the red blood corpuscles. If we grant that these premises are correct, then any agent capable of bringing about a sufficient increase in the number of the red corpuscles becomes of therapeutic value. We have been unable to obtain details of the records to which reference has been made. If secretin is to exert any influence as an immunizing agent by increasing the number of red corpuscles in the circulating blood, it is obvious that a single dose must be capable of causing a great and fairly prolonged rise in the red corpuscle count. As a means of ordinary treatment, hypodermic medication is preferable to intravenous, and if it can be shown that secretin administered hypodermically is able to increase the number of red corpuscles, then again, in order to be of service, a single dose, or at most three or four successive doses, should produce and maintain a largely increased erythrocyte count.

Acting in accordance with the ideas thus suggested we determined to try first, the effect of a certain arbitrarily fixed dose of secretin given intravenously and second, the effect of the same dose when introduced hypodermatically.

In our selection of the animal to be used we were guided by the recent work of Lamson (9) on acute polycythemia in which he has shown that adrenalin, fright, pain, etc., cause sudden and very marked elevation of the red corpuscle count in the dog and cat but that these agents are without effect on the erythrocyte count of the rabbit. Therefore, that we might avoid the use of an anaesthetic, especially in those experiments which were to be continued over several days, in order that the attending conditions might be as uniform as possible, rabbits were employed in all of the experiments recorded in this paper.

The secretin was in all cases prepared from the intestine of the dog. The animal was anaesthetized by ether alone and the upper half of the small intestine removed. This intestine was carefully washed in running water and the mucous membrane scraped off with a dull knife. The scrapings were rubbed up in a mortar with sand, covered with 50 cc. of 0.4 per cent hydrochloric acid and allowed to stand for an hour or more. The mixture was then boiled actively for several minutes, neutralized with strong potassium hydroxide while boiling and again rendered faintly acid with glacial acetic acid. Finally the preparation was strained through muslin and filtered.

We found that this preparation when kept in the dark retained its

potency for about five days; but if glacial acetic acid were added to the filtrate in sufficient quantity to make this 2 per cent acid by volume and the solution evaporated to dryness, the residue was found to retain its potency for months at least. When required, a weighed quantity could be dissolved in distilled water and neutralized, thus giving a preparation of the same effectiveness as the original solution.

Over two hundred determinations of the red corpuscles per cubic millimeter of blood were made in the course of these experiments, the blood being obtained from the ear of the rabbit and the count made in the usual manner with the Thoma-Zeiss apparatus.

The dose of secretin solution selected for the first experiments was 1 cc. per kilogram of body weight. Five rabbits were taken, the erythrocytes per cubic millimeter of blood counted, and the proper dose of secretin injected into the femoral vein. The results of these experiments are recorded in table 1.

TABLE 1

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
1	5,320,000	7,510,000	2,190,000	41.16	30	60
2	4,560,000	6,990,000	2,430,000	53.29	15	70
3	4,450,000	6,350,000	1,900,000	42.69	40	65
4	5,610,000	8,210,000	2,600,000	46.35	45	90
5	4,830,000	5,630,000	800,000	16.56	25	45
Averages....	4,954,000	6,938,000	1,984,000	40.04	31	66

As a type of this series of experiments the first one is given in detail:

Experiment 1, November 6, 1916

10.05 a.m. Red blood corpuscles, 5,320,000 per cubic millimeter
10.10 a.m. 1 cc. secretin per kilogram of body weight given intravenously
10.25 a.m. Red blood corpuscles, 6,940,000 per cubic millimeter
10.40 a.m. Red blood corpuscles, 7,510,000 per cubic millimeter
10.55 a.m. Red blood corpuscles, 7,120,000 per cubic millimeter
11.10 a.m. Red blood corpuscles, 5,350,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 5,290,000 per cubic millimeter

The next thing to be determined was the effect of the same dose upon the number of red corpuscles when it was introduced beneath

the skin. A tabulated report of the results obtained in this way will be found in table 2.

TABLE 2

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
6	5,120,000	6,305,000	1,185,000	23.1	60	90
7	4,548,000	5,844,000	1,296,000	28.4	30	30
8	4,536,000	5,545,000	1,009,000	22.2	30	90
9	4,906,000	5,619,000	713,000	14.5	55	95
10	5,840,000	7,197,000	1,357,000	23.2	60	90
Averages....	4,990,000	6,102,000	1,112,000	22.2	47	79

As typical of this series of experiments the first one is here presented in detail:

Experiment 6, November 20, 1916

1.30 p.m. Red blood corpuscles, 5,120,000 per cubic millimeter
1.35 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.05 p.m. Red blood corpuscles, 5,336,000 per cubic millimeter
2.35 p.m. Red blood corpuscles, 6,305,000 per cubic millimeter
3.35 p.m. Red blood corpuscles, 5,601,000 per cubic millimeter
4.35 p.m. Red blood corpuscles, 5,006,000 per cubic millimeter

A comparison of the results obtained in these two groups of experiments shows certain features in favor of the intravenous method of administration. The most striking difference is in the percentage increase—an average of 40 per cent when the secretin is given intravenously and 22.2 per cent when given hypodermatically. However, if we note the average actual increase in number of red corpuscles the difference is only slightly over one-half million in favor of the intravenous method—1,984,000 in the former case and 1,112,000 in the latter. As might be expected the intravenous method gives the effect in a shorter time than the subcutaneous,—the maximum effect obtained in thirty-one minutes in one instance and in forty-seven minutes in the other; but when we compare the duration of the effect we find it almost identical in the average of the two groups—sixty-six minutes was the average time that the increase lasted when the secretin was given intravenously and seventy-nine minutes when it was given hypodermatically.

The second group of experiments had convinced us that secretin injected subcutaneously was capable of exerting an influence, at least so far as affecting the number of red corpuscles in circulation was concerned. Moreover, the greater effect obtained by giving the secretin intravenously was not sufficiently pronounced to make it the method of choice so far as any therapeutic application was concerned. Therefore, we decided to adhere to the method of hypodermic administration in the remainder of our experiments, particularly as these two series of observations appeared to furnish sufficient data from which to deduce the probable action of any particular dose of secretin when given intravenously if we had determined its effect when given hypodermatically.

To determine the most effective dose we made several series of experiments using the following doses per kilogram of body weight: 0.75 cc., 0.5 cc., 0.25 cc., 1.5 cc., 2 cc. In this way we tested the effect of amounts of secretin less than and greater than the original and arbitrary dose of 1 cc. per kilogram of body weight. Each group table gives the summarized results of each experiment in the group and the averages for that particular series. Following each group summary is a report giving the details of one experiment in the group, serving as an example of all.

TABLE 3

Dose: 0.75 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
13	5,327,000	6,224,000	897,000	16.8	60	90
14	6,334,000	7,168,000	834,000	13.1	30	30
20	6,384,000	7,098,000	714,000	11.0	60	60
21	5,324,000	6,961,000	1,637,000	30.7	90	90
Averages....	5,842,250	6,862,750	1,020,500	17.9	60	67.5

Experiment 13, January 12, 1917

9.30 a.m. Red blood corpuscles, 5,327,000 per cubic millimeter
10.20 a.m. 0.75 cc. secretin per kilogram of body weight given hypodermatically
10.50 a.m. Red blood corpuscles, 5,206,000 per cubic millimeter
11.20 a.m. Red blood corpuscles, 6,224,000 per cubic millimeter
12.20 p.m. Red blood corpuscles, 6,042,000 per cubic millimeter
1.20 p.m. Red blood corpuscles, 5,100,000 per cubic millimeter

TABLE 4

Dose: 0.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
16	6,251,000	6,837,000	586,000	9.3	30	30
19	5,741,000	7,875,000	2,134,000	37.1	90	90
24	6,048,000	No effect				
30	5,804,000	No effect				
31	6,760,000	7,046,000	286,000	4.2	60	60
Averages....	6,120,800	6,722,000	601,200	10.1	36	36

Experiment 16, January 22, 1917

12.55 p.m. Red blood corpuscles, 6,251,000 per cubic millimeter
1.00 p.m. 0.5 cc. secretin per kilogram of body weight given hypodermatically
1.30 p.m. Red blood corpuscles, 6,837,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 6,079,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 6,426,000 per cubic millimeter

TABLE 5

Dose: 0.25 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
11	5,888,000	6,810,000	922,000	15.6	30	30
17	5,754,000	No effect				
18	5,280,000	6,144,000	864,000	16.3	30	30
25	4,075,000	5,015,000	940,000	23.1	30	30
27	5,970,000	No effect				
Averages....	5,395,000	5,940,200	545,200	11.0	18	18

Experiment 11, November 28, 1916

1.10 p.m. Red blood corpuscles, 5,888,000 per cubic millimeter
1.15 p.m. 0.25 cc. secretin per kilogram of body weight given hypodermatically
1.45 p.m. Red blood corpuscles, 6,810,000 per cubic millimeter
2.15 p.m. Red blood corpuscles, 5,988,000 per cubic millimeter

TABLE 6

Dose: 1.5 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
12	6,376,000	7,440,000	1,064,000	15.1	60	90
22	7,188,000	No effect				
28	4,959,000	7,162,000	2,203,000	44.8	120	120
32	5,936,000	7,416,000	1,480,000	24.9	60	90
Averages....	6,114,750	7,301,500	1,186,750	21.2	58	60

Experiment 12, December 20, 1916

10.00 a.m. Red blood corpuscles, 6,376,000 per cubic millimeter
10.30 a.m. 1.5 cc. secretin per kilogram of body weight given hypodermatically
11.00 a.m. Red blood corpuscles, 6,937,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 7,440,000 per cubic millimeter
12.30 p.m. Red blood corpuscles, 6,014,000 per cubic millimeter

TABLE 7

Dose: 2 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
15	4,112,000	5,188,000	1,076,000	26.1	30	60
23	5,928,000	6,935,000	1,007,000	16.9	30	90
26	5,858,000	6,471,000	613,000	10.4	30	90
29	5,400,000	7,502,000	2,102,000	38.9	60	120
Averages....	5,324,500	6,524,000	1,199,500	22.4	37.5	90

Experiment 15, January 12, 1917

9.20 a.m. Red blood corpuscles, 4,112,000 per cubic millimeter
9.30 a.m. 2 cc. secretin per kilogram of body weight given hypodermatically
10.00 a.m. Red blood corpuscles, 5,188,000 per cubic millimeter
10.30 a.m. Red blood corpuscles, 4,507,000 per cubic millimeter
11.30 a.m. Red blood corpuscles, 4,207,000 per cubic millimeter

As we proceeded with our observations the results pointed to a dose of 1 cc. per kilogram of body weight as being the most efficient. In order to assure ourselves on this point we made seven more experiments in which the dose was 1 cc. per kilogram of body weight. Taken to-

gether with the five original experiments recorded in table 2 we have a total of twelve such determinations. For the purpose of computing an average with as large a number of experiments as possible these have been brought together in table 8.

TABLE 8

Dose: 1 cc. secretin solution per kilogram of body weight

EXPERIMENT NUMBER	INITIAL COUNT	MAXIMUM COUNT	AMOUNT OF INCREASE	PERCENTAGE INCREASE	MAXIMUM IN	DURATION OF EFFECT
					minutes	*minutes*
6	5,120,000	6,305,000	1,185,000	23.1	60	90
7	4,548,000	5,844,000	1,296,000	28.4	30	30
8	4,536,000	5,545,000	1,009,000	22.2	30	90
9	4,906,000	5,619,000	713,000	14.5	55	95
10	5,840,000	7,197,000	1,357,000	23.2	60	90
33	5,778,000	6,894,000	1,116,000	19.3	30	90
34	5,872,000	6,579,000	707,000	12.0	50	70
35	6,200,000	6,850,000	650,000	10.4	50	90
36	5,456,000	5,955,000	499,000	9.1	70	80
37	5,200,000	7,240,000	2,040,000	39.2	25	105
38	4,720,000	7,264,000	2,544,000	53.8	75	30
39	5,684,000	6,752,000	1,068,000	18.7	35	30
Averages....	5,321,666	6,503,666	1,182,000	22.2	47.5	73.3

Reviewing the average percentage increase with each dose we find the results to have been as follows: 0.25 cc. secretin per kilogram of body weight, 11.0 per cent; 0.5 cc. secretin per kilogram of body weight, 10.1 per cent; 0.75 cc. secretin per kilogram of body weight, 17.9 per cent; 1 cc. secretin per kilogram of body weight, 22.2 per cent; 1.5 cc. secretin per kilogram of body weight, 21.2 per cent; 2 cc. secretin per kilogram of body weight, 22.4 per cent. These records indicate a dose of 1 cc. per kilogram of body weight as the most efficient dose of secretin. We also find that the longest average time the effect lasted was ninety minutes—where the dose was 2 cc. secretin per kilogram of body weight. With a dose of 1 cc. per kilogram of body weight the average duration was 73.3 minutes. It seemed worth while to test the effect of repeated doses of secretin on the increase in the number of erythrocytes per cubic millimeter of blood, both as to the amount of increase and the duration of the increase. Is it possible to produce a summation effect? To answer this question the following experiments were performed: One in which a dose of 1 cc. secretin per kilogram of

body weight was followed in two hours by a second dose of 1 cc. per kilogram of body weight; two experiments in each of which four successive doses of 1 cc. secretin per kilogram of body weight were administered at intervals of one hour; one experiment in which five successive doses of 1 cc. secretin per kilogram of body weight were given at intervals of one hour; one experiment in which five successive doses of 1 cc. secretin per kilogram of body weight were given at intervals of twenty-four hours. The results of these observations are appended.

Experiment 40, February 14, 1917

9.45 a.m. Red blood corpuscles, 4,548,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.30 a.m. Red blood corpuscles, 5,844,000 per cubic millimeter
11.00 a.m. Red blood corpuscles, 5,338,000 per cubic millimeter
12.00 noon Red blood corpuscles, 5,025,000 per cubic millimeter
12.30 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
1.00 p.m. Red blood corpuscles, 5,042,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 5,600,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 5,787,000 per cubic millimeter

February 15, 1917

10.00 a.m. Red blood corpuscles, 4,286,000 per cubic millimeter

February 16, 1917

10.00 a.m. Red blood corpuscles, 4,457,000 per cubic millimeter

Experiment 41, February 19, 1917

9.55 a.m. Red blood corpuscles, 5,765,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.55 a.m. Red blood corpuscles, 7,094,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.55 a.m. Red blood corpuscles, 6,756,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
12.55 p.m. Red blood corpuscles, 7,459,000 per cubic millimeter
1.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.00 p.m. Red blood corpuscles, 8,327,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 6,749,000 per cubic millimeter
4.00 p.m. Red blood corpuscles, 6,195,000 per cubic millimeter

February 20, 1917

9.00 a.m. Red blood corpuscles, 6,156,000 per cubic millimeter

February 21, 1917

9.00 a.m. Red blood corpuscles, 5,832,000 per cubic millimeter

Experiment 42, February 20, 1917

8.55 a.m. Red blood corpuscles, 5,245,000 per cubic millimeter
9.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
9.55 a.m. Red blood corpuscles, 5,645,000 per cubic millimeter
10.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.55 a.m. Red blood corpuscles, 6,723,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.55 a.m. Red blood corpuscles, 6,659,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
1.00 p.m. Red blood corpuscles, 6,384,000 per cubic millimeter
2.00 p.m. Red blood corpuscles, 5,553,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 5,284,000 per cubic millimeter

Experiment 43, February 21, 1917

10.00 a.m. Red blood corpuscles, 5,825,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
10.30 a.m. Red blood corpuscles, 6,522,000 per cubic millimeter
10.55 a.m. Red blood corpuscles, 6,764,000 per cubic millimeter
11.00 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically
11.30 a.m. Red blood corpuscles, 6,004,000 per cubic millimeter
11.55 a.m. Red blood corpuscles, 7,402,000 per cubic millimeter
12.00 noon 1 cc. secretin per kilogram of body weight given hypodermatically
12.30 p.m. Red blood corpuscles, 8,883,000 per cubic millimeter
12.55 p.m. Red blood corpuscles, 6,111,000 per cubic millimeter
1.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
1.30 p.m. Red blood corpuscles, 5,472,000 per cubic millimeter
1.55 p.m. Red blood corpuscles, 7,016,000 per cubic millimeter
2.00 p.m. 1 cc. secretin per kilogram of body weight given hypodermatically
2.30 p.m. Red blood corpuscles, 7,094,000 per cubic millimeter
3.00 p.m. Red blood corpuscles, 8,089,000 per cubic millimeter
4.00 p.m. Red blood corpuscles, 6,912,000 per cubic millimeter

February 22, 1917

10.00 a.m. Red blood corpuscles, 5,446,000 per cubic millimeter

February 23, 1917

10.00 a.m. Red blood corpuscles, 5,296,000 per cubic millimeter

Experiment 44, February 22, 1917

10.00 a.m. Red blood corpuscles, 5,829,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 23, 1917

10.00 a.m. Red blood corpuscles, 5,245,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 24, 1917

10.00 a.m. Red blood corpuscles, 7,710,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 25, 1917

10.00 a.m. Red blood corpuscles, 6,961,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

February 26, 1917

10.00 a.m. Red blood corpuscles, 5,523,000 per cubic millimeter
10.05 a.m. 1 cc. secretin per kilogram of body weight given hypodermatically

March 1, 1917

10.00 a.m. Red blood corpuscles, 5,975,000 per cubic millimeter

March 4, 1917

10.00 a.m. Red blood corpuscles, 6,218,000 per cubic millimeter

These experiments, numbers 40 to 44 inclusive, show that successive doses of secretin at short intervals are capable of causing a progressive increase in the number of red corpuscles per cubic millimeter of blood, but the increase is not maintained from one dose to the next; so that between the doses there is a diminution from the maximum count resulting from that dose before the next dose exerts an effect. In other words, two doses do not give twice the effect of one dose or three doses three times the effect of one dose. Moreover, when the administration of the secretin is stopped the number of red corpuscles in the circulating blood reverts to normal almost as quickly as after a single dose. In the case of the rabbit which received a daily dose of secretin for five days the increase in the number of erythrocytes per unit volume of blood on the eighth day as compared with the initial count was 146,000, showing that secretin has no ability to produce a permanent increase in the red corpuscle content of the circulating blood of the normal animal.

Three main conclusions are inevitable from the observations that have been recorded: first, secretin, even when injected subcutaneously, is capable of producing an increase in the number of red corpuscles in the circulating blood; second, the increase thus effected is not great as compared with the increase that may be obtained by the action of other agents; third, the length of time that this larger number of red corpuscles persists is comparatively short.

Let us consider briefly the possible therapeutic benefits that may be derived from the exhibition of secretin. If we grant the correctness of the theory of Beveridge and Williams (10) that the red corpuscles constitute one of the chief defensive agencies of the animal organism against the invasion of pathogenic bacteria or the products of such bacteria, then, in order that aid may be given to the establishment of immunity by this means, we must be able in some way to bring about a marked augmentation in the number of red corpuscles and an augmentation that will continue for a time sufficiently long to be of service. We have shown that the most efficient dose of secretin is in the proportion of 1 cc. per kilogram of body weight, which means for the average man 70 cc. of secretin subcutaneously or 38.5 cc. intravenously. Furthermore the effect of this dose disappeared on an average 73.3 minutes after it was given. Moreover, it was not possible to produce a lasting increase in the number of red corpuscles by giving successive doses, either at intervals of one hour, two hours or twenty-four hours. The facts that have been adduced militate against any therapeutic value for secretin but do not detract in the slightest from its physiological significance. On the contrary, they appear to give secretin added importance in the normal organism. It is possible that one of the means by which the normal number of red corpuscles is maintained in the blood stream is the action of secretin.

Naturally the next question that presents itself is: How does secretin produce this increase in the number of the red corpuscles in the circulating blood? This is a question which we are not as yet prepared to answer, but several suggestions can be offered. The first and simplest explanation that presents itself is that secretin exerts a direct stimulating influence upon the red marrow of the bones thus leading to the formation of new cells. Such a conclusion is entirely in accord with the known activities of secretin. If this substance is capable of promoting the activity of the pancreatic cells, of the hepatic cells, and of the cells of the pituitary body, it is entirely reasonable to assume that it may also have the ability to increase the formation of red blood corpuscles by the red marrow of the bones.

A second way in which secretin might bring about an increase in the number of circulating erythrocytes is by causing variations in their unequal distribution. This might be effected by a direct constricting action on the capillaries of some large area, such as the liver, or by an indirect action through stimulation of the adrenals. Lamson (9) has shown that in the cat and dog an increase in the number of red cor-

puscles per unit volume of blood may be obtained by the administration of adrenalin. In the same animals fright raises the number of red corpuscles per unit volume of blood an average of 80 per cent. In both cases there is no increase in the number of erythrocytes if the hepatic artery be ligated. It has been shown by Cannon (11) that fright stimulates the adrenals, and Lamson attributes the presence of a greater number of red corpuscles in the blood stream to a constriction of the capillaries of the liver caused by adrenalin. Mautner and Pick (12) inform us of the presence of an extremely sensitive nervous mechanism in the liver of the dog, reacting to epinephrin by constriction of the capillaries, and the absence of such in the liver of the rabbit, or the presence in this animal of a much less sensitive mechanism. Lamson (13) has shown also that excitement or the intravenous injection of adrenalin causes no polycythemia in rabbits. Therefore, it seems that we can rule out the suggestion that secretin increases the number of red corpuscles in the circulating blood of the rabbit by stimulating the adrenals. As to whether the secretin acts directly to promote capillary constriction or not we have no evidence and do not know of any work that has been reported on the subject.

One other explanation that should be mentioned is the possibility that secretin causes a decrease in plasma volume and thus gives rise to a higher erythrocyte count per cubic millimeter of blood.

The means by which secretin acts to produce an increase in the number of red corpuscles in a unit volume of blood is a question outside of the scope of the present investigation. In undertaking these experiments we were actuated by the desire to know whether secretin increased the number of erythrocytes in the blood stream or not; and, if so, how much of an increase could be hoped for, and how long it would be possible to maintain this increase. These questions have been answered, we believe, and the solution of the mode of action will be found later.

CONCLUSIONS

1. It is possible to produce a considerable increase in the red corpuscle count per cubic millimeter of blood by the administration of secretin even in small doses and by subcutaneous injection.

2. The most efficient dose is 1 cc. of secretin per kilogram of body weight.

3. The increase in the count appears quickly and is very transient.

4. By repeating the dose of secretin at short intervals the increase in the erythrocyte count can be kept up for several hours but drops promptly after the administration of the last dose.

5. The administration of secretin over a period of five days, in daily doses of 1 cc. per kilogram of body weight, has very slight, if any, lasting effect on the red corpuscle count in the normal animal.

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THE ACTION OF ULTRA-VIOLET RADIATION IN KILLING LIVING CELLS SUCH AS BACTERIA

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(From experiments carried out at Nela Research Laboratory)

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Several theories have been advanced in an attempt to explain the mode of action of ultra-violet radiation in killing living cells. One theory is that the short wave lengths of the spectrum act by destroying the intracellular enzymes. So far as I have been able to find, the only basis for this theory is the fact that intracellular enzymes in common with other enzymes are destroyed by exposure to ultra-violet radiation. The object of this investigation is to show that the destruction of living cells, such as bacteria, by ultra-violet radiation is not due to the destruction of the intracellular enzymes but to the coagulation of the protoplasm of the cells by the radiation.

The bacteria used were B. liquefaciens, B. prodigiosus, B. fluorescens, B. proteus vulgaris, B. pyocyaneus and B. subtilis. These bacteria were chosen because they possess the property of liquefying gelatine, this property in turn being dependent upon the intracellular proteolytic enzymes. Twenty-five cubic centimeters of liquid containing great numbers of B. liquefaciens were exposed in an open vessel to the radiation from a quartz-mercury vapor burner, operating at 140 volts, 3.3 amperes, at a distance of 10 cm., until they were dead as was indicated by negative results on plating. By means of a centrifugalizing machine the dead bacteria were thrown down and subsequently ground up in a mortar with sand and 30 per cent alcohol. In this way the intracellular enzymes were extracted from the dead bacteria. All of the bacteria named above were treated after this manner. Ten cubic centimeters of the alcoholic extract of the different kinds of dead bacteria were introduced into separate test-tubes containing gelatine. Ten cubic centimeters of liquid containing the different kinds of living bacteria were also introduced into tubes containing gelatine. These tubes were permitted to stand at room temperature

for ninety-six hours. At the end of this time the extent to which the gelatine had been liquefied in the different tubes was measured. The measurements are given in table 1.

TABLE 1

Under the different kinds of bacteria are shown the extent of liquefaction of gelatine by the living bacteria and by extracts of the dead bacteria

BACTERIA	LIQUEFACIENS	PRODIGIOSUS	FLUORESCENS	PROTEUS VULGARIS	PYOCYANEUS	SUBTILIS
	mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
Extent of liquefaction by living bacteria.....	12	8	6	6	5	4
Extent of liquefaction by extract of dead bacteria...	10	7	6	5	4	4

It may be seen that the gelatine in the tube containing living B. liquefaciens was liquefied 12 mm.; that containing living B. prodigiosus, 8 mm.; B. fluorescens, 6 mm.; B. proteus vulgaris, 6 mm.; B. pyocyaneus, 5 mm.; and B. subtilis, 4 mm. It may also be seen that the extract of dead B. liquefaciens had liquefied 10 mm. of gelatine; B. prodigiosus, 7 mm.; B. fluorescens, 6 mm.; B. proteus vulgaris, 5 mm.; B. pyocyaneus, 4 mm.; and B. subtilis, 4 mm. If the amount of gelatine liquefied by the living bacteria be compared with that liquefied by the extract of the corresponding dead bacteria, it will be found that there is very little difference in the extent of liquefaction. This is taken to mean that, while the ultra-violet rays had killed the bacteria, it had affected very little their intracellular enzymes. These experiments would seem to render untenable the theory that ultra-violet rays kill living cells by destroying their intracellular enzymes.

The following experiments were carried out to show that ultra-violet radiation kills living cells by coagulating their protoplasm. Several drops of water containing great numbers of paramecia were introduced into a shallow glass vessel and covered with a quartz plate. The glass vessel was then placed on a block of ice under a quartz mercury vapor burner operating at 140 volts, 3.3 amperes, at a distance of 5 cm., and in this position the organisms were exposed for twenty minutes. A drop of the liquid containing the dead paramecia was mixed with a drop containing living ones on a glass plate and covered with a cover glass. Having located under a microscope a dead organism and a living one lying close together a micro-photograph was

made of them. Similarly micro-photographs were made of paramecia killed by heating to 45°C. and 90°C. respectively. These photographs are shown in figure 1. The upper organisms under *A*, *B* and *C* are living transparent animals; the lower one under *A* was killed by heating to 90°C., the lower one under *B* by heating to 45°C. and the lower one under *C* by exposure to ultra-violet radiation. By comparing the lower organisms under *B* and *C* it may be seen that there is no difference in the appearance of these two organisms, both being slightly more opaque than the living organisms. The lower organism under *B* was killed by the coagulation of its protoplasm by heat and since there is no difference in the appearance between this one and the lower

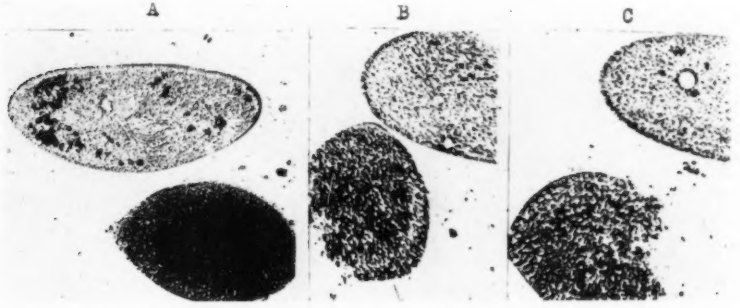

Fig. 1. Microphotographs of paramecia. The upper ones under *A*, *B* and *C* are the normal transparent living animals; the lower one under *A* was killed by heating to 90°C.; the lower one under *B* by heating to 45°C; the lower one under *C* by exposure to ultra-violet radiation.

organism under *C*, which was killed by exposure to ultra-violet radiation, it would seem to be fair to assume that the latter was killed by the coagulation of its protoplasm by the radiation. By comparing the lower organism under *A* with that under *B* it may be seen that the lower one under *A* is very much more opaque than the lower one under *B*. This greater opacity is explained by the fact that proteins are more firmly coagulated at a temperature of 90°C. than at a temperture of 45°C. It will be noticed also that the lower organisms under *B* and *C* which were killed by heating to 45°C. and by exposure to ultra-violet radiation respectively had begun to disintegrate while the lower one under *A* had not begun to do so because of the firmer coagulation of the protein of this organism heated to the higher temperature.

Henri (1), Hertel (2) and others observed that when protozoa were exposed to ultra-violet radiation the body became swollen, water drops appeared on the surface and the organisms finally disintegrated, but they did not observe any coagulation produced by the radiation. The failure of these observers to obtain conspicuous coagulation in the organisms was due to the fact that the radiation to which they exposed the organisms was not of sufficient intensity to coagulate firmly the protoplasm. If paramecia are heated to 40°C. they are killed after a time, but very little indication of coagulation is produced as is indicated by the fact that there is very little decrease in the transparency of the organisms thus killed. By increasing the temperature, however, at which the organisms are killed, the protoplasm becomes firmer and the animals more opaque. Similarly by killing the organisms by exposure to ultra-violet radiation of low intensity, a very inconspicuous amount of coagulation is produced, and hence there is very little change in the transparency of the organisms. If the intensity of the radiation is increased, however, the coagulation of the protoplasm, as well as the opacity of the animals, becomes more marked.

CONCLUSION

Exposure of living cells to ultra-violet radiation of sufficient intensity to kill the cells does not decrease to any appreciable extent the activity of the intracellular enzymes.

Evidence is presented in this paper to show that ultra-violet radiation kills living cells by coagulating their protoplasm.

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THE EFFECT OF THYROID FEEDING ON THE CATALASE CONTENT OF THE TISSUES

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It has been observed that when animals are fed thyroid or an extract of the gland they lose weight and strength. The increased oxygen intake and carbon dioxide output of such animals show that oxidation is augmented while the increased nitrogen elimination indicates an increased tissue destruction. Magnus-Levy (1) observed an increased carbon dioxide output in a man fed upon thyroid extract and an increased oxygen intake in cases of exophthalmic goiter. Fritz Voit (2) found that thyroid feeding increased protein metabolism in dogs. Anderson (3) observed a decrease in metabolism in cases of myxedema as was indicated by a decreased oxygen intake and carbon dioxide output, and that the metabolism was increased to normal by thyroid feeding. As a result of these and similar observations it is generally accepted that the effect of thyroid feeding or of hypersecretion of the glands as in exophthalmic goiter is to increase oxidation and tissue destruction, while in myxedema there is a decrease in metabolism. It has been observed that when oxidation is increased or decreased in a tissue the catalase content is correspondingly increased or decreased (4). Since thyroid feeding causes an increase in oxidation a corresponding increase in catalase should be found if the relationship between oxidation and catalase is to hold. It has also been observed that when the catalase content of a tissue is decreased the tendency of that tissue to undergo autolysis is correspondingly increased (5). Since thyroid feeding increases autolysis in muscular tissue, for example, as is indicated by a loss in weight and strength of the muscles, there should be a corresponding decrease in catalase in the muscles and in all other tissues in which autolysis is increased. It is known that the autolyzing enzymes in common with all the ordinary enzymes are easily oxidized and destroyed.

The object of this investigation was to determine if thyroid feeding increases the catalase content of certain tissues, which would account for the increased oxidation in animals fed thyroid, while in other tissues, such as the muscles and fat, it causes a decrease in oxidation which would account for the increased autolysis in these tissues. The animals used in these experiments were cats. They were placed in separate cages and fed twice a day 5 grams of desiccated thyroid mixed with 60 grams of ground meat. It was found necessary to vary the diet frequently by mixing the thyroid with different kinds of meat in order to induce the cats to eat. Fresh white fish, canned salmon, beef sausage and liver were among the meats used. The control or normal cats were fed the same kinds and amounts of food as the thyroid cats except that there was no thyroid added to their food. Even with every inducement it was found that certain cats refused to eat after the first day or two. The data given in this paper were obtained from cats that had eaten thyroid for at least five days. After this period of feeding the cats were used as soon as they refused to eat or when they showed great emaciation. None of the animals were fed thyroid longer than two weeks.

After etherizing the cats approximately 25 cc. of blood were collected from each cat and allowed to clot. The blood vessels of the animals were then washed free of blood by the use of large quantities of 0.9 per cent sodium chloride at 38°C., as was indicated by the fact that the wash water gave no test for catalase. The heart, the back (latissimus dorsi, trapezius) and leg (biceps, semi-membraneous) muscles were removed and ground up in a hashing machine. The clotted blood was pressed through several thicknesses of cheese cloth and ground up in a mortar. The catalase content of the muscles was determined by adding 1 gram of the hashed muscle to 45 cc. of hydrogen peroxide in a bottle and as the oxygen gas was liberated it was conducted through a rubber tube to an inverted burette previously filled with water. The volume of gas was read off directly from the burette where it had displaced the water. After reducing this volume to standard atmospheric pressure the resulting volume was taken as a measure of the catalase content of the gram of material. In determining the catalase content of the blood, 10 drops of blood were added to 500 cc. of hydrogen peroxide in a large bottle and as the oxygen gas was liberated it was conducted through a rubber tube to a large inverted graduated cylinder previously filled with water, After reducing the volume of gas which was read off directly from the cylinder to stand-

ard atmospheric pressure, the resulting volume was taken as a measure of the amount of catalase in the 10 drops of blood. The hydrogen peroxide used in all these determinations was prepared by diluting commercial hydrogen peroxide with an equal volume of distilled water. It was found very necessary to use the same make of hydrogen peroxide in all the determinations since the different makes gave different results. For this work a stock supply of about 200 liters of hydrogen peroxide was purchased and kept in a container in a dark and cool place. The results of the determinations for the blood and heart of the normal and the thyroid-fed cats are given in table 1.

TABLE 1

After blood and heart are given the number of cubic centimeters of oxygen liberated in ten minutes from hydrogen peroxide by 10 drops of blood and 1 gram of hashed heart respectively

	CAT										AVERAGE AMOUNT OF OXYGEN
	1	2	3	4	5	6	7	8	9	10	
											cc.
Blood											
Normal cat	560	430	970	1040	640	720	630	630	750	890	726
Cat fed thyroid	1280	2285	1850	1520	1940	2220	2560	2200	1570	1460	1888
Heart											
Normal cat	210	219	228	248	228	220	228	212	204	213	221
Cat fed thyroid	154	126	162	198	198	184	190	160	115	120	161

It may be seen in the table that the average amount of oxygen liberated from 500 cc. of hydrogen peroxide in ten minutes by 10 drops of blood of the normal cats was 726 cc.; that of the cats fed thyroid, 1888 cc. of oxygen. The average amount of oxygen liberated by 1 gram of the hashed heart of the normal cats was 221 cc. of oxygen, while the average for the hearts of the cats fed thyroid was 161 cc. From these results it is evident that thyroid feeding increased the catalase content of the blood by approximately 160 per cent as is indicated by the increase from 726 cc. to 1888 cc. of oxygen, while it decreased the catalase content of the heart by approximately 30 per cent as is indicated by the decrease from 221 cc. to 161 cc. of oxygen. The catalase content of the leg and back muscles and in some cases of the fat was determined, but the results were not very uniform and for that reason they were not included in the table. On the whole, however, it might be said that the catalase content of these tissues and hence oxidation was probably decreased by thyroid feeding.

It has been shown that the catalase content is an index to the amount of oxidation in a tissue, being greatest where the amount of oxidation is greatest and least where oxidation is least. From this it follows that thyroid feeding increases oxidation in the blood and decreases it in the heart and probably in the fat and skeletal muscles. Furthermore, it has been observed that when oxidation is decreased in a tissue the tendency of that tissue to undergo autolysis is increased. This was shown in starvation, for example, where oxidation is decreased in the skeletal muscles and fat, with a corresponding increase in the rate of autolysis resulting in the carrying into solution of these less vital tissues, while oxidation in a more vital organ, such as the heart, remains normal, with a corresponding high resistance to autolysis. The mechanism by which thyroid feeding produces a loss in skeletal muscles and fat would seem to be the same as that which causes the loss in starvation, namely increased autolysis made possible by decreased oxidation in these tissues. The effect of starvation on the heart, however, is different from that of thyroid feeding in that starvation does not decrease oxidation in this organ while thyroid feeding does, with the resulting increase in autolysis. This decreased oxidation with resulting increase in the rate of autolysis of the heart may explain the harmful effect on the heart encountered in thyroid feeding for obesity. It may also account for the characteristic heart disturbances in exophthalmic goiter where there is a hypersecretion of the thyroid glands. The increased oxidation in animals to which thyroid is fed may be accounted for by the great increase in catalase and hence in oxidation in the blood.

Many more cats were used in this investigation than the numbers indicate in the table. Most of these were the animals that refused to eat the thyroid after a day or two of feeding. Some of these animals were used as soon as they refused to eat, while others were kept and starved until they began eating again. The results from these cats indicated that thyroid feeding began to decrease the catalase in the heart after about two days, while it required four or five days' feeding to increase the catalase content of the blood.

CONCLUSIONS

1. Thyroid feeding increases the catalase of the blood and decreases it in the heart and probably in the fat and skeletal muscles.

2. The increased catalase of the blood may account for the increased oxidation in animals to which thyroid is fed, while the decreased cata-

lase in the heart, skeletal muscles and fat may account for the increased autolysis in these tissues, the idea being that when oxidation is decreased in these tissues a smaller amount of the autolyzing enzymes is oxidized and destroyed, resulting in an increase in the rate of autolysis.

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CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH NO. 67

RHEOTROPIC RESPONSES OF EPINEPHELUS STRIATUS BLOCH

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CONTENTS

I. INTRODUCTION

A. The problem

The tropical fish known as hamlet or grouper (*Epinephelus striatus* Bloch) is very favorable for biological experimentation. It is easily obtained and is a hardy animal; in response it is deliberate, but definite. When considerable numbers of hamlets are held in confinement they often manifest a tendency to crowd together closely, though without any regular arrangement, their heads pointing in all directions. This characteristic may have helped to give them the common name of "grouper."

While engaged in a study of the reactions of this fish, in connection with certain work on its central nervous system, I made use of an apparatus of the following nature: In a large spawning trough (fig. 1, *A*), such as is used in fish hatcheries, I suspended by two supports (*B*, *B*) a cage (*D*), about 36 inches long by 18 inches wide and 10 or 12 inches deep, made of galvanized "chicken wire." A current of fresh sea water was introduced at one end of the cage, at an angle of about 30° with the horizon, by a 1-inch hose pipe, for the purpose of affording a

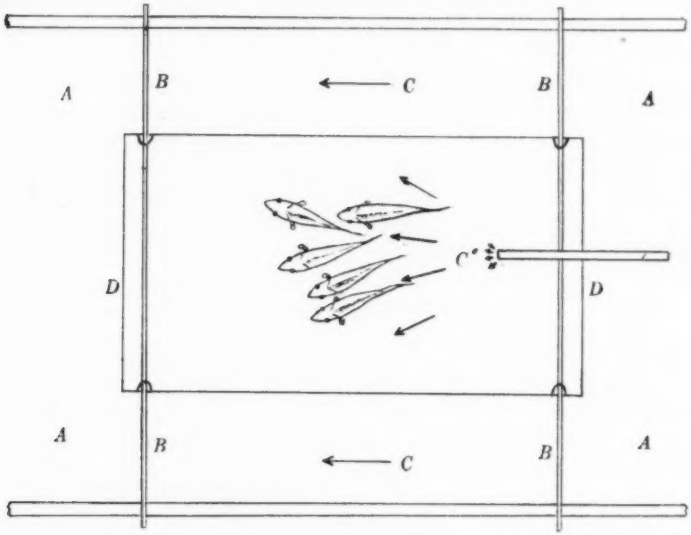

Fig. 1. Diagram of cage as seen from above. *A*, Large spawning trough; *B*, rods supporting cage; *C*, current of spawning trough; *C'*, additional currents from hose pipe; *D*, wire cage suspended from *B*.

better supply of water than was furnished by the sluggish current of the trough, flowing in the same direction.

When several normal fishes had been put in the cage for temporary storage, it was noticed that their orientation was no longer promiscuous, as in quiet water, but that an unusual position was assumed by nearly all of them (fig. 1). The cause of this was not at once apparent; but a little experimentation showed that the new orientation was in response to the stimulus of the seawater delivered through the hose pipe. Contrary to the usual rheotropic response of fishes, they had

their heads directed *away* from the hose pipe, most of the time with the body axis in line with the current, so that a group of fishes showed a fanlike arrangement corresponding to the spreading currents of water *C*, as shown by the arrows in figure 1.

In order to determine the exact nature of this reaction, experiments were undertaken both on groups of fishes and on individuals. The study of groups showed that this peculiar orientation was not altogether constant, but that some of the fishes assumed from time to time positions more or less oblique to the current. The precise significance of this was manifest only when the actions of individual fishes were observed continuously.

In an effort to ascertain the precise mechanism by which these reactions were brought about, and if possible to determine the nature of the stimulus inducing them, a small but strong localized current of water was directed in succession against different areas of the body. This revealed varying sensitivity on different areas; furthermore the effect of a narcotic on these areas indicated both the position and the probable nature of the end organs involved in these responses.

B. Review of literature

Up to about fifteen years ago it was generally held that the orientation and locomotion of organisms in a current of water—whereby the anterior end is directed against the current and a swimming motion causes either an advance or the maintenance of a comparatively stationary position against the flow—was due to the direct mechanical action of the current and was in the nature of a reaction to pressure. Stahl in 1884 described such a phenomenon in Myxomycetes and Verworn (1) in his Allgemeine Physiologie (p. 428) interprets it as a positive response to pressure stimulation. Lyon (2) (p. 157) says that reactions of blinded fishes [Fundulus?] to currents flowing through troughs may *perhaps* be caused "by higher pressure on one part than on the other, through differences in the velocity of the water striking the two parts." He here introduces the theory of unequal pressures on various parts of the fish's body in contrast with "the gross mechanical one of Radl."

The one early exception to the theory of pressure stimulation by currents seems to have been the idea advocated by Schulze (3), that the lateral-line organs were stimulated by the movement of water against them. This view has, however, been adequately disproved by Parker (4).

In the same year Tullberg (5) (p. 20) carried out experiments in which he eliminated the ear of certain fishes and found the operated animals to be insensitive to water currents. He therefore concluded that the ear is the chief receptor of this stimulus, which in his opinion affects principally the cristae acusticae of the ampullae. Parker (6) (pp. 202–203) contended, on the other hand, that the failure of fishes to orient in a normal fashion to the current when their ears had been destroyed is caused by an interference with the ear, "though the primary stimulus for this form of response might be received by the skin."[1]

In confirmation of this view—i.e., primary cutaneous sensitivity to currents—he (4) (p. 61) showed that specimens of Fundulus heteroclitus in which the lateral-line nerves had been severed responded normally,—i.e., swam against the current in a glass tube,—and he (6) (p. 202) also succeeded, after cutting of the lateral-line nerves and the spinal cord (in an anterior region), in inducing normal responses to a current of water directed against the sides of the body posterior to the cuts. Moreover, he excluded the possibility of stimulation through the ear or lateral-line senses by severing the appropriate nerves and draws this conclusion (4) (p. 63): "Surface waves and current action" . . . . "must stimulate the general cutaneous nerves (touch)."

However, his attempts to inhibit the action of the cutaneous nerves by the application of cocaine, and thus to show directly what the indirect method (the elimination of ear and lateral-line organs) had rendered probable, were unsuccessful.

In 1904 Lyon, basing his conclusions upon the results of ingenious experiments with Fundulus, scup, stickleback and butterfish surrounded by movable environments, put forward another and totally new explanation of rheotropism. He contended that "the primary cause of orientation [and locomotion of fishes] in streams of some uniformity of motion is an optical reflex." "The essential element of stimulation is the environment [apparently moving, but in reality stationary], not the currrent;" the latter "does not directly stimulate." He, however, adds that cutaneous sensations [touch?]—contact of the body with stationary solid objects and even "the sliding contact between fish and [rushing] water"—may sometimes be the cause of such orientation and locomotion. But even these responses, like those of a strictly optical reflex nature, are to be regarded, he thinks, as

[1] i.e., stimulation of the skin (tactile corpuscles) directly by small currents. This, it should be noted, is different from the indirect (optical reflex or thigmotropic—by solid object) stimulation, which is described below.

compensating motions; "the current playing only the passive part of sweeping the fish against objects on the bottom."

In view of Lyon's observations and general conclusion that the current itself does not stimulate the skin of Fundulus directly, except as it acts like a solid object of considerable size, it seems desirable to determine whether, in other fishes, the integument is sensitive to water-currents, and also whether the eyes have any essential part in the rheotropic reactions.

II. DESCRIPTION OF EXPERIMENTS

A. Posterior[2] and lateral orientation

1. In groups of fishes. The positions assumed by a group of fishes under the conditions already described gave, in general, the fanlike appearance shown in figure 1; but it was also to be seen that, from time to time, one or more individuals assumed a different position, the body swinging around so that its long axis was almost or quite perpendicular to the current; and sometimes, though rarely, an individual would be headed more or less directly into the current in the manner of the hitherto described reactions of fishes generally. In order to test roughly the quantitative relations at any given instant between these various positions, several series of observations were made, both during the day and at night, on a group of seven fishes which had become habituated to their surroundings. One such series is recorded in table 1; all the others are nearly identical with it.

From table 1 it will be seen (1) that, although the majority of fishes (67.1 per cent) tailed into the current, many individuals (31.9 per cent) so placed themselves that the current hit the side of the body; and (2) that less than 1 per cent headed into the current. These records were taken at about two-minute intervals. While they show that the hamlet in its normal responses to a current may assume one or the other of two positions, they do not afford sufficient evidence of

[2] In describing the various positions of orientation which a fish may assume, I shall use *posterior* orientation to denote positions in which the tail is directed toward the oncoming current or to points not more than 45 degrees from it on either side; *lateral* orientation to denote positions in which the long axis of the body is perpendicular to the direction of the current or makes an angle with the perpendicular on either side not greater than 45 degrees; and *anterior* orientation to positions whereby the head is directed straight into the current or to points not more than 45 degrees from it on either side.

TABLE 1

Successive positions of orientation of a group of seven hamlets observed at about two minute intervals

	NUMBER OF INDIVIDUALS IN EACH OF THE THREE POSITIONS AT THIRTY SUCCESSIVE COUNTS																													TOTAL	AVERAGE	PER CENT
Number of the observation...	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	210	7	100
Orientation																																	
1. Posterior...	6	6	4	3	5	6	5	5	4	4	5	5	5	5	6	5	6	5	4	6	7	5	2	3	5	2	3	5	4	5	141	4.7	67.1
2. Lateral.....	1	1	2	4	2	1	2	2	3	3	2	2	2	2	1	2	1	2	3	1	0	2	4	4	2	5	4	2	3	2	67	2.2	31.9
3. Anterior....			1																				1								2	0.06	0.9

the exact nature of this reaction—whether the two positions are due to differences between individual fishes or are phases of one reaction. Single groupers, however, when studied continuously showed that both positions are assumed in the course of one reaction.

2. In individual fishes. Individual hamlets were tested in a spacious oblong aquarium (fig. 2) provided with plane glass front and back to allow uninterrupted observation from the sides as well as from above. A glass tube 1 cm. in diameter and so directed as to make about equal angles with two adjacent sides of the aquarium, delivered a strong current (*C*) diagonally across the tank. The volume of this current was approximately 0.1 liter per second. It is the only one which is significant for the purposes of this investigation. Those peripheral to this were not of sufficient strength or regularity to influence the fish in any consistent manner. These currents were studied by the use of floating and suspended objects, and the results plotted show their main features.

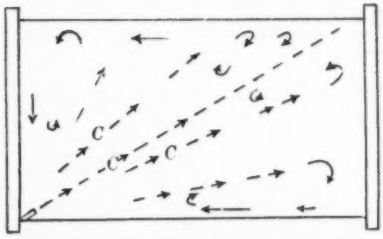

Fig. 2. Diagram of aquarium (20 by 30 inches) and currents as seen from above. *C*, Main diagonal current.

The fish under investigation (fig. 3) seemed to prefer the region of the strongest current; that is, it remained near the source of the current, shifting from one position of quiet to another, settling to the bottom, or remaining suspended at a fixed place in the current for a few moments, and then again changing position slightly. All the while, however, the fish assumed either posterior or lateral orientation to the

main diagonal current (*C*). It held this position in the tank indefinitely. When left in the current for two or three hours no change of general position was noted. When the current was shut off, the fish under investigation would swim to the bottom or to one of the corners of the tank, where it would remain relatively quiet. In figure 3, one out of several records is reproduced to show a typical reaction. The diagonal line (*C*) shows the direction of the main current. The elapsed time in minutes, from the beginning of observations, is given in table 2 for each of twelve successive positions. These positions are indicated in figure 3 by straight lines, the anterior end of the fish being denoted by a short cross line, and the successive positions by consecutive numerals placed near the end representing the tail.

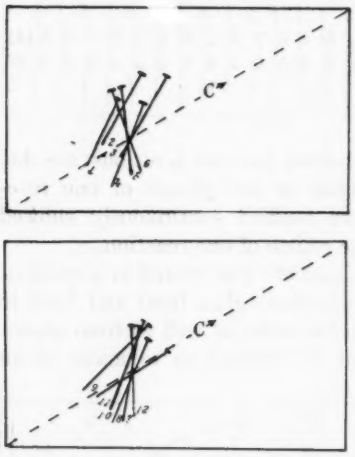

Fig. 3. Twelve successive positions occupied by a normal fish with reference to the main diagonal current, *C*. To avoid confusion only six positions are indicated on each of the two diagrams. Position 11 was retained longer than any other; it is therefore regarded as the most significant orientation. One complete reaction is regarded as occupying the interval of time between two successive assumptions of such a position.

It should be noted, however, that in this particular experiment, contrary to the most of them, the percentage of posterior and lateral orientations is nearly equal. That posterior orientation is the purpose of shifting the position, is suggested by the fact that the fish remained stationary for a considerable time only when it was almost directly tail into the current, position 11. After this period (about two minutes) it again begins a series of changes in position, like those shown in figure 3, which lasts for about four minutes, whereupon another period of rest ensues. What I have called one complete reaction, then, requires about seven minutes. It is most important to note that the fish did not at any time head into the current.

The two different experiments, one with groups and the other with individuals, are consistent in showing that posterior and lateral orientation to a current is the normal reaction of *Epinephelus striatus*.

TABLE 2

Time elapsed in assuming the positions, 1 to 12, shown in figure 3

	NUMBER OF THE POSITION											
	1	2	3	4	5	6	7	8	9	10	11	12
Elapsed time in minutes	0	½	¾	1	1¼	1½	2	3	3½	3¾	4¾	7
Time intervals between positions in minutes		½	¼	¼	¼	¼	½	1	½	¼	1	2¼

B. Experiments on regional sensitivity

A study of the relative sensitivity of various parts of the body was undertaken with the hope of finding some evidence as to the nature of the causes which produce this peculiar rheotropic reaction. The method employed to determine the sensitivity of different areas of the surface of the fish to a current was to direct a jet of water at close range against limited areas (approximately 25 sq. cm.) and to note the response, if any. For this purpose a long glass tube with a bore 1 cm. in diameter was used, and the average flow of water through it was roughly 1/28 liter per second. In this way all parts of the dorsal and lateral surfaces were explored. To prevent as far as possible any complication that might result from sight stimuli, the observer was screened from the fish by a curtain covering the side of the tank. The curtain was provided with a hole for observation; the tube was nearly invisible. In all cases the local current stimulation produced a negative reaction, a swimming or backing away from the current. It is possible, however, to divide the body into five regions based on their relative susceptibility to stimulation by such a current. In the order of promptness of reaction these are as follows: lip region (seven seconds); caudal fin (sixteen seconds); dorsal fin, posterior part (twenty-two seconds);[3] cheek and operculum (twenty-five seconds); sides of body (about thirty seconds). The belly was not tested because of its inaccessibility. Thus it appears that the lip region is by far the most sensitive part of the integument tested. If stimulation of the lips is prolonged, the hamlet becomes very vigorous in its attempts to escape.

[3] That the fins are not essential in rheotropism is indicated by the fact that when either dorsal or caudal fins are removed, the normal reaction is unaltered. It was also noticed that fishes whose fins had become badly frayed by long captivity were normal in their responses to currents shown in figure 3.

When "cornered" by the current it literally stands on its head, a termination of the negative reaction which is extremely unusual among fishes.[4] This high sensitivity suggests at once an explanation of posterior and lateral rheotropism, for it may be that stimulation of the lips by the current in the aquarium or cage was so strong as to produce decided irritation, and thus to cause the fish to place that portion of its skin in a less exposed position.

C. Summary of normal rheotropism

The foregoing experiments show:

1. That to a moderate artificial water-current a normal orientation of *Epinephelus striatus*, in groups or individually, is posterior or lateral, as phases of one complete reaction, but almost never anterior.
2. That the lips are the most sensitive integumentary region, other regions being less sensitive in the following order; tail>, dorsal fin>, side of head>, middle of body.
3. That the peculiar posterior and lateral reaction to a current is perhaps an attempt to protect from the current the highly sensitive lips.

D. The end organs concerned in rheotropism

1. Method of determination. In searching for the end organs concerned in rheotropism, it was, of course, necessary to consider all possible sensory cells. My conclusions relative to the significance of equilibration (semi-circular canals), muscle sense, and pressure sense in these reactions are, for the most part, based upon observations only. The lateral-line organs, eyes and cutaneous receptors, on the other hand, were experimentally eliminated, and each operated fish was carefully studied to detect any resulting variation of the response from that of the normal individual. It was established by these experiments that the end organs concerned in rheotropism in the hamlet are located in the integument and are probably the tactile corpuscles.

2. Observations and experiments: a. Observations. When confined in large volumes of still water, groupers are seen usually to lie inactive on the bottom of the tank. In captivity they swim about very little, seem-

[4] It is important to note that the same reaction can be induced by the use of a fine glass rod (tactile stimulation), and also that the variation in regional sensitivity to such stimulation corresponds exactly to that described for stimulation by the current.

ing to prefer muscular repose to exertion, the fin movements being few and slow; pectoral fins are vibrated about twenty-three times per minute. The application of a localized current of little force was sufficient to start the fishes from a position of complete rest, but when they were beyond the range of the current, they again settled to the bottom. This behavior indicates that the agreeableness of muscular effort is not sufficient to cause any prolonged swimming. On the other hand, the exertion and possible fatigue involved in maintaining a relatively constant position in a current which is broad enough to cover the whole fish might be expected eventually to produce a negative reaction. Instead of this, however, the fishes remained for an hour or two in the strongest part of the current (fig. 3), seeming to prefer it to the quiet water. This leads one to the conclusion that the muscular effort necessitated in these reactions is not in itself a deterrent factor. Many times, too, when a localized current was directed against the side of the fish, lying at the bottom near the wall of the aquarium, it was observed that one of the pectoral fins—extended horizontally to the wall—served to keep the body of the fish from contact with the side of the tank. In these cases the effort involved in maintaining this position did not cause the reaction time to vary.

With a view to ascertaining what importance, if any, attaches to the pressure sense, I made use of a current of water directed through the glass tube (1 cm. in diameter) already referred to in other experiments. If the pressure sense is a factor in rheotropic response, it is to be expected that the response to a very strong current would be more prompt than to a weaker one. Accordingly I repeatedly subjected the same fish successively to a weak current (about 1/28 liter per second) and to a stronger one (about 1/8 liter per second). Though the latter was of sufficient force to produce an appreciable indentation of the skin and musculature of the body, the reaction time was not shorter than in the case of the weaker current. I may also add that fishes can be pressed by the hand against the side of the aquarium with considerable force without causing any definite response. In his study of the pressure sense as a possible cause of rheotropism, Lyon (2) (p. 154) enclosed fishes (silver sided minnows) in long stoppered bottles which were floating down the stream. He found that under these conditions, with all pressure stimulation thus eliminated, the fishes responded normally, by swimming in a direction opposite to the drift of the bottle, in an attempt to keep the visual environment constant. My experiments, though not of such fundamental importance as Lyon's,

tend to substantiate his conclusion that [considerable] pressures do not cause or influence the rheotropic reactions.

Observations were also made upon the equilibration of hamlets. They were studied in still water and when subjected to a current sufficiently strong to cause a change in the direction of the dorso-ventral axis. Any differences between the rheotropic response of fishes whose dorso-ventral axis is normal and those in which this axis has been displaced would suggest that the organs of equilibrium may be involved. An individual fish when in quiet water usually lies in such a position that its dorso-ventral axis makes an angle of 10° to 15° with the normal (fig. 4). When this angle was doubled by a current from a glass tube directed against the side of the fish there was no variation in the time required to produce a negative reaction. Moreover the motion which restores the fish to an approximately vertical position usually follows, and never precedes, this rheotropic response; whereas, if the fish is carefully put in the same oblique position by a slow displacement with the hand, instead of by the current, the righting movement takes place much more promptly. It seems, then, that any slight disturbance in the hamlet's equilibrium which the current might cause would neither produce nor in any way affect the observed behavior. This conclusion is in accord with Parker's conclusion (6) (p. 203) to which reference has already been made.

b. Experiments. Lateral-line organs can be excluded from the list of possible rheotropic receptors for two reasons: First, when the current is directed immediately against the lateral-line canal upon a limited mid-body area, the slow response (thirty seconds) characteristic of regions both dorsal and ventral to the lateral-line, but excluding it, is neither quickened nor retarded. Secondly, a hamlet in which all of the lateral-line nerves had been severed responded normally to the current. This experiment confirms Parker's results (4) (6) from a similar test made upon Fundulus.

In order to determine whether the visual organs are essential to these reactions, experiments were also performed successively on several individuals after enucleation of both eyes. Fishes thus blinded were subjected to conditions of stimulation identical with those in the tests which were made upon unoperated hamlets (fig. 2) and the successive positions which they assumed were recorded. One of these records is reproduced (fig. 5, table 3) for comparison with figure 3, which shows the consecutive orientations of a normal fish in a like environment.

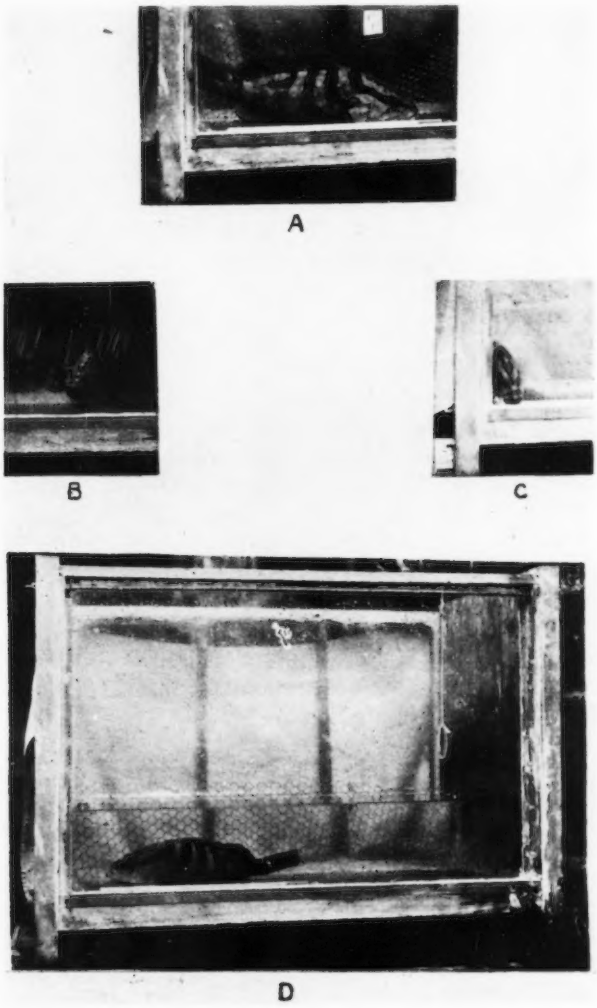

Fig. 4. Photographs of resting fishes tipped at a characteristic angle from the vertical. *A*, Side view; *B* and *C*, front views; *D*, side view of fish, showing the whole aquarium.

The two records, while showing slight variations, are remarkably alike in the time of response and the number of different positions assumed in changing from an almost lateral orientation to an approximately posterior one. It should be noted that in this series of orientations 75 per cent of the positions were posterior, and that, as in figure 3, no anterior positions were assumed. It seems, then, that the eyes of the hamlet are not the essential rheotropic end organs.

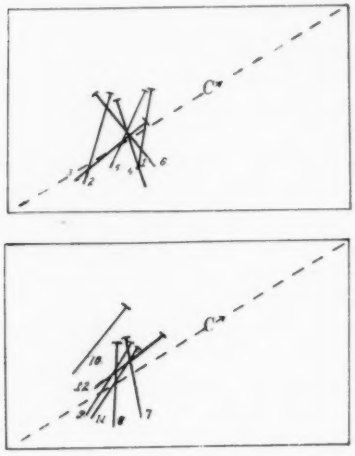

Fig. 5. Twelve successive positions assumed by a blinded fish in response to the current *C*. As in figure 3, only six positions are shown in each diagram.

In an effort to locate the cells which are stimulated by the current, the skin was stripped from one of the more sensitive body areas. It was impossible to obtain any response from a current which was directed against the subcutaneous structures (muscles, etc.) thus exposed. In a few cases indefinite reactions, which were much slower than normal, were observed, but they were not characteristically rheotropic in nature. These results lead one to the conclusion that the end organs concerned in rheotropism are located in the integument.

TABLE 3

Time elapsed in assuming positions 1–12 (fig. 5)

	NUMBER OF THE POSITION												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Time elapsed in minutes.....	0	½	¾	1	1¼	1½	2	2½	3	3½	4	4½	7
Time intervals between positions in minutes.....		½	¼	¼	¼	¼	½	½	½	½	½	½	2½

The problem of determining the particular type of sense organ which is sensitive to these currents is resolved, then, into a physiological

study of the hamlet's cutaneous end organs. Of these only the tactile corpuscles are significant, because the receptors for chemical, photic and thermal stimuli plainly can not be involved in these rheotropic responses.

It has been stated previously that the areas of greatest cutaneous sensitivity in the case of both touch and current stimulation have the same distribution. Cocaine is known to inhibit the functioning of the end organs of touch. It was, therefore, used to eliminate, functionally, the tactile corpuscles, in order that their relation to rheotropism might be determined. Of all available areas the lips were chosen for the application of this reagent because the experiments had shown that their stimulation gave the most marked and peculiar responses. Whether the lips are stimulated by a fine glass rod or by a moderate water current, the fish performs the very curious reaction of either backing away violently or of standing on its head. The method of treatment with the cocaine was as follows: the fish was removed from the experiment tank and the lips were immediately immersed in a 0.1 per cent solution of sulphate of cocaine for about ten seconds; this was supplemented by bathing the lips with the same solution applied by means of a soft cloth at about ten-second intervals for fifty seconds. After this treatment the fish was returned to the tank and its responses to stimulation by a glass rod and by a weak current (about 1/28 liter per second) were noted. By a repetition of the treatment it became evident that there was a slowing down in time of response with increased exposure to the solution and that this was precisely the same for both types of stimulation. Two repetitions of the first treatment—in all about three minutes—were usually sufficient to inhibit completely all responses to either type of stimulation; neither the glass rod nor the localized current then produced any reaction. In subsequent trials the lips were treated continuously—without periodic subjection to stimulation—for a period of about three minutes. The effect was the same as in the preliminary treatment just described. After such administration of cocaine the fish swam about in a slightly abnormal manner, manifesting an irritation due, doubtless, to the drug. This insensitivity of the lips lasted about a minute, sometimes a few seconds longer. Then a gradual functional recovery occurred until, at the end of three to four minutes, normal responses could be obtained by the use of either stimulus. It is most significant that the time of disappearance of normal sensitivity, as well as that of its reappearance, was absolutely the same for both kinds of stimulation.

This fact indicates that those sensory cells which are stimulated by touch and defunctionized by cocaine are also the cells which are the primary end-organs of the rheotropic response.

3. Summary of end-organ determination. 1. The end organs of the hamlet essentially concerned in rheotropism are located within the integument.

2. The regional distribution of sensitivity to a water-current and to touch is the same.

3. Cocaine applied to the lips for about three minutes renders those organs insensitive both to touch and to currents.

4. These facts indicate that the end organs of touch serve also as the essential end organs of current stimulation in the hamlet.

5. Other sensory cells may be more or less affected by currents in some fishes, but they appear to be only accessory end-organs of rheotropism, and in the responses described in this paper they evidently play no part at all.

III. DISCUSSION

There is much evidence to show that the rheotropic responses of the hamlet, as suggested for Fundulus by Parker (4) (6), are effected chiefly by the tactile corpuscles. His attempt to prove this by immersing the entire fish in a solution of cocaine did not succeed because the general action of the drug entirely destroyed all sensitivity and movements of the fish; but the great sensitivity of the lips of the hamlet has afforded an excellent opportunity to study changes in the fish's behavior resulting from the local application of this narcotic. In this case regional anaesthesia, producing insensitivity to touch, is as satisfactory as general narcotization would be, because it causes a most unique rheotropic response totally to disappear.

Some of Lyon's experiments (2) on rheotropism, from which he concluded that the reaction of Fundulus to currents is chiefly to compensate the transporting effect of the current, seem to furnish evidence that the integumentary cells (tactile corpuscles) were directly concerned in the rheotropism which he observed. Among these is experiment 9 (p. 157), in which a blinded fish, without touching any solid object—often required for orientation by fishes without eyes—headed into the rushing current. This certainly may be interpreted as a response to direct tactile stimulation of the integument by the water, and Lyon himself admits that this may be called a *true* rheotropism. In his opinion, however, it is due to the "sliding contact" (stereotropism)

between fish and water, although he admits the possibility of another interpretation involving the idea of unequal pressures on different parts of the body of the fish. Whether the rheotropism induced by this "sliding contact" is the equivalent of stereotropism, with which Lyon believes it is closely related, is a question needing further investigation.[5]

The comparative importance, in the behavior of Fundulus, of these two types of impressions—optic and cutaneous—is, I think, suggested by some of Lyon's interesting experiments. An example of this is his experiment 3 (p. 153). Here a normal fish, surrounded by a rapidly moving artificial environment, is immersed in a current of water flowing in the same direction as the environment, but less rapidly. The fish swims in the direction of, but faster than, the current flow, following the moving environment in rate as well as direction. This is unquestionably a case of optical response. When the environment stops moving, the current still flows on in the same direction, but with a gradual decrease of speed due to friction; but the fish, having been carried passively by the current, turns, without a reference point, and faces [swims against?] the current. This may be due, as Lyon says, to an apparent reversal of the visual field; but, since *blinded* fishes (experiment 9), *without* touching a reference point, also orient against the current, it seems equally logical to interpret the turning of fishes with eyes (experiment 3) as the result of the normal rheotropic response to direct tactile stimulation of the integument, which had, during the movement of the optical field, been subordinated to the sight-reflex.

If this be a proper interpretation of the results with Fundulus, we have in the hamlet a reversal of the relative importance of the two kinds of stimuli. Here, under the experimental conditions described, all optic stimuli were, apparently, subordinated to tactile impressions; the direct effect of the current being predominant and able to cause entirely normal reactions in the absence of eyes. It is, however, not quite satisfactory to compare the two sets of experiments (those by Lyon and by myself) from this point of view, because the relative amounts of cutaneous and optic stimuli in each are indefinite and variable. It is certain that, in my experiments, there was relatively little stimulation of the eyes, because the fish remained in an approximately

[5] It seems probable that the currents of a narrow trough would not be sufficiently strong nor distinct from one another to simulate solid objects, but that there would be an almost insensible gradation between them.

constant position, and that, in Lyon's movable-environment experiments, their total stimulation was much greater. It can not be said whether the tactile corpuscles were subjected to a proportionate stimulus or not. It may be that the hamlet, too, would orient to a movable environment regardless of contemporaneous tactile stimulations[6] by the current; but the facts remain that the tactile-corpuscles do, of themselves, effect orientation, and that this orientation under the above circumstances is unaltered by the presence of eyes. This orientation, it seems to me, is caused by a direct stimulation of the integument by the water currents as such, and to it we should apply the term rheotropism. The response so well described by Lyon as due to optic reflex might then be called a rheoscopic response in view of the fact that it is due to the optical effect of a flowing or moving environment.

How the current stimulates these tactile end organs is still a matter of speculation. It may be that differences of velocity in different portions of a current provide slight local variations of force sufficient to cause a definite response on the part of the fish. How the stimulation results in orientation is a further question, for the mechanism and sensation may or may not be the same for rheotropism that they are for stereotropism.

The author wishes to express his appreciation to Dr. E. L. Mark for the privilege of working at the Bermuda Biological Station, and to Dr. Mark and Dr. W. J. Crozier for valuable assistance and advice in the work.

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[6] It would be interesting to determine the relative importance of the eyes and cutaneous elements (tactile corpuscles) as sense organs in the orientation and rheotropic motions of many other fishes.

FURTHER EVIDENCE REGARDING THE RÔLE OF THE VAGUS NERVES IN PNEUMONIA

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A former communication[1] recorded the discovery "that section of the vagus nerves protects the respiratory cells and prevents their exhaustion in pneumonia. In pneumonic dogs in which both vagus nerves have been cut, the rate of respiration remains normal throughout the disease. In such animals there is no dyspnoea."

It was necessary in that research to cut the right vagus nerve within the chest and to obtain complete recovery from that operation before cutting the left vagus nerve in the neck. Some days after this second operation the dogs were inoculated with pneumonia. The disease ran its usual course, except for the extraordinary fact that the typical dyspnoea was entirely absent. Obviously, the section of the vagus nerve within the chest and the recovery of the animal without infection are troublesome and time-consuming procedures. This method is essential to the demonstration that the exhaustion[2] of the respiratory mechanism characteristic of pneumonia does not take place when the path of afferent impulses from the lungs to the bulbar respiratory cells is severed by the section of the vagi. It is not, however, essential to the demonstration that the dyspnoea in pneumonia depends on impulses passing from the lungs through the vagus nerves. The dependence of the dyspnoea upon vagal impulses may be shown by cocainizing the vagi while the pneumonia is at its height. The following protocol is evidence of this.

Experiment June 15, 1916. At 4 p.m., 24 cc. of a broth culture of Friedländer's bacillus were injected into the right bronchus of an anaesthetized dog weighing 8 kilos.

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[1] Porter and Newburgh: This Journal, 1916, xlii, 175.

[2] Discovered by Newburgh, Means, Porter: Journ. Exper. Med., 1916, xxiv, 583.

June 16, 9 a.m. Rectal temperature, 40°C. Respiration labored; 80 per minute. The dog was placed on the operating table and lightly but sufficiently etherized. The vagus nerves were exposed in the neck and were surrounded by a layer of absorbent cotton.

The absorbent cotton was wet with a 1 per cent solution of cocain.

9.30 a.m. Respiration, quiet, "easy," 20 per minute.
10.00 a.m. Respiration, 16.
12.00 m. Respiration, 16; temperature, 40°.
1.00 p.m. Respiration, 15.
2.00 p.m. Respiration, 30.
3.00 p.m. Respiration, 60; temperature, 40°.
3.15 p.m. A few drops of cocain solution were dropped on the cotton surrounding the vagus nerves.
3.30 p.m. Respiration, 16.
5.00 p.m. Respiration, 14; temperature, 39° (the beginning of the fatal fall).
6.00 p.m. Respiration, 14; temperature, 37.5°. The dog lies upon his side; he cannot stand.
6.30 p.m. Respiration, 14; temperature, 37°. Dog semi-conscious.
7.00 p.m. Respiration, 14; temperature, 37°. Dog in coma.
7.10 p.m. Death.

Autopsy. Red hepatization of the right middle and both lower lobes of the lungs.

CONCLUSION

Cocainizing the vagus nerves changes the violent dyspnoea of pneumonia into quiet, normal breathing.

OROKINASE AND SALIVARY DIGESTION STUDIES IN THE HORSE[1]

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INTRODUCTION

The work reported in this paper may be divided into four main groups or parts, as follows:

I. Orokinase—an enzyme produced by the glands in the mouth, which activates the saliva.

II. Bacteria of the mouth as activating agents.

III. Amylolytic action of mixed saliva obtained from the mouth and esophagus.

IV. The amount of complete starch digestion in the mouth.

The work was planned and outlined, the tissue extracts prepared and the operations were performed upon the experimental horses by Palmer. Anderson carried out the bacteriological studies and assisted in the operations and preparation of the tissue extracts. Malcomson studied the amounts of starch conversion in the mouth. Peterson studied the amylolytic action of mixed saliva. The entire group participated in the studies with the activating substances.

I. OROKINASE

Orokinase is the name proposed by Palmer for the enzyme produced in the mouth and found in the saliva, which makes active the inert saliva emptied into the mouth from the salivary glands. We believe, and our experiments prove, that this enzyme, orokinase, is produced by the buccal glands, and possibly by the lingual glands.

[1] Published with the approval of the Director as Paper No. 65 of the Journal Series of the Minnesota Agricultural Experiment Station.

That we should find such an activating substance was suspected before we began our studies. The idea was presented through a study of the literature. Ellenberger and Hofmeister (1) state that mixed saliva of the horse has a very powerful diastatic action, whereas saliva collected from the parotid ducts is inactive. Mathews (2) in reviewing this fact suggests the possibility of a co-ferment or kinase produced by the mucous membrane of the mouth. He also adds that "the late Dr. Cook told the writer that if the human mouth is carefully washed out with a sterile solution of water or dilute antiseptic, the saliva collected from the ducts may be inactive, whereas the saliva which has been in contact with the mucous membrane of the mouth is very active."

Our first work, then, consisted in confirming Ellenberger and Hofmeister's work, and especially since some workers do not accept this view but are of the opinion that horse saliva is inactive under all conditions. Why these investigators have failed to demonstrate the amylolytic activity of mixed horse saliva, will be discussed under part III.

We have been able to confirm Ellenberger and Hofmeister's statement that saliva is inactive before it reaches the mouth, by studying the amylolytic activity of parotid fistula saliva of three horses, and the glycerine or water extracts of the parotid, submaxillary and sublingual glands of eight horses. The same methods of study were employed as those used by Palmer (3) in his studies on ox saliva.

Basing our conclusions on approximately one hundred examinations of the substances named, we conclude parotid fistula saliva when stimulated under natural conditions (feeding oats) is without trace of amylolytic activity upon cooked or raw starch at least within a period of several hours incubation. Glycerine and water extracts of the three salivary glands are also without action as indicated by clearing of the starch solution, loss of blue color with iodine, or the presence of reducing sugars.

We also agree with Ellenberger and Hofmeister that mixed saliva is very powerful. This conclusion is based upon the study of seventeen positive cases. In eleven horses active mixed saliva was collected from the mouth, and in six it was obtained from the esophagus. The details of this work are discussed in part III.

Another method of demonstrating that mixed saliva is very powerful, is by feeding raw corn and oats; these substances contain no reducing sugars before feeding, but show heavy reduction when caught from an esophageal fistula. This work is discussed in part IV.

From this work it is evident that in the mouth the previously inactive saliva becomes active, and our work was now directed towards locating this activating substance. Ellenberger and Hofmeister suggested bacteria of the mouth as the activating agents, but we were unable to confirm this, as shown in our bacteriological studies. Our efforts to locate the activating substance were first rewarded on December 7, 1916, when on this date a 50 per cent glycerine and water extract of the mucous membrane of the buccal region gave excellent results. Only a few drops of this extract were required to activate parotid fistula saliva and gland extracts from the three salivary glands. If we used a large enough quantity of this extract, it would not only activate the fistula saliva and gland extracts, but it would digest starch itself. We could, however, so reduce the amount until we had a quantity sufficient to activate the fistula saliva or gland extracts, but which would not alone digest starch within a period of two hours incubation.

Further studies with this activator "I" as we called it, revealed the following facts: Its ability to activate the saliva was destroyed by boiling. When a few drops of this activating substance were added to 50 cc. of fistula saliva, and incubated for several hours, we could not demonstrate that a small amount could activate an indefinite amount of fistula saliva if given time enough.

Our next step was to demonstrate this activating substance in a number of animals. The next three buccal mucous membrane extracts gave negative results, so we began to search anew for the activating substance. In carefully dissecting the mouth, the small buccal glands are found under the mucous membrane of the cheeks and lips, and many of the lobes are quite adherent to the mucous membrane. In fact it is difficult to dissect the mucous membrane away from its underlying parts without removing some of these glands with it. These glands form quite a large mass, just anterior to the commissures in the lower lip, and another large group is found in the buccal region just posterior to the commissures. In addition to these locations, the glands are numerous under the mucous membrane of the buccal region, and here two rows of small ducts present themselves. In some subjects there is a small deposit of dark pigment around the opening of each duct, and the ducts number about one hundred or more. The glands are also quite numerous under the mucous membrane of the lower lip and the openings of their ducts can be easily made out. In a fresh specimen a small quantity of the secretion of these glands can be squeezed out through the ducts and onto the mucous membrane where

it can be collected. In two specimens this buccal juice would activate fistula saliva, would not digest starch itself, and was destroyed by heat.

We have now demonstrated in ten horses that extracts of the buccal glands contain the activating substance. In several of these cases the mucous membrane from the buccal region and the lips was carefully removed, but they were negative in all cases. Our results in the first case (activator I) can probably be accounted for by the fact that some of these glands were removed with the mucous membrane. The activators obtained from these ten cases have not all behaved alike. Some of them would digest starch as well as activate fistula saliva; some would activate the saliva, so that the digestion was carried to the maltose stage, while others would only carry the digestion to the soluble starch or dextrin stage. They were all destroyed by heat.

Our best extracts have been obtained from horses freshly killed, but even some of these were unstable and lost their activating power after thirty-six to forty-eight hours. For example, the most powerful activator (No. III) was obtained from a horse a few hours after death; this activator was very powerful and table 1 shows in detail one experiment using this activator, but after standing twenty-four hours this extract was inactive. This activator would not digest starch when used alone and was destroyed by heat.

We did not succeed in obtaining a potent extract in every case examined. We invariably failed in cases where the horse had been dead for a few days or when the head had been frozen, and in our positive cases the activators seemed to vary in strength, even though we tried to use the same relative amount of extracting material in each case. This may be accounted for by the fact that the activator is unstable, or our methods of extracting are not ideal, or that after death substances are present in the buccal glands which destroy this activating substance.

These buccal glands and the openings of their ducts are located and pour out their secretion in places which are well adapted to activate saliva coming into the mouth. The parotid duct in the horse opens opposite the third upper cheek tooth, and as this parotid saliva flows down over the buccal mucous membrane, it directly comes in contact and mixes with the secretion of the buccal glands. The ducts of the submaxillary gland opens opposite the canine tooth, and here in this region just anterior to the commissure of the lips we find another group of glands very similar in gross structure to the buccal and very likely belonging to the same class. Similar glands are also found under the

TABLE 1

Detail of one experiment demonstrating the action of orokinase on fistula saliva and salivary gland extracts

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Nearly clear	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	Fair	More	Heavy
B_s	Nearly clear	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
C_s	Considerable	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
D_s	Considerable	Clear	Clear	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Fair	More	Heavy	Heavy
E_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
F_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
G_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
H_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
I					No clearing					Blue					No reduction
J					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
B_s contains: Parotid gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
C_s contains: Submaxillary gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
D_s contains: Sublingual gland extract, 1 cc.; buccal extract, 0.5 cc.; starch, 5 cc.
E_s contains: Fistula saliva, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
F_s contains: Parotid gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
G_s contains: Submaxillary gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
H_s Contains: Sublingual gland extract, 1 cc.; distilled H_2O, 0.5 cc.; starch, 5 cc.
I contains: Buccal extract, 0.5 cc.; distilled H_2O, 1.0 cc.; starch, 5 cc.
J contains: Distilled H_2O, 1.5 cc.; starch, 5 cc.

mucous membrane of the lower lip, and the secretion of these glands mixes with the sublingual saliva.

The lingual glands on the base of the tongue may also produce orokinase, since mixed saliva coming from the esophagus is more powerful than any mixed saliva which we have obtained from the anterior part of the mouth. Extracts of the lingual glands (located in the base of the tongue) have been prepared from five horses, and in all cases the extract alone would digest starch either to the soluble starch, dextrin or maltose stage, but they did not activate fistula saliva.

We have succeeded in procuring from the mouth of one animal what we believe to be almost pure buccal juice. A grey mare which normally was a willing animal to salivate, had a parotid fistula on the right side. We would stimulate secretion by teasing with oats and corn and from the right cheek obtain a secretion much more viscid than the fistula saliva and which resembled buccal juice.[2] We know this was not parotid saliva because it did not appear like parotid secretion and because no parotid saliva was being poured out onto this cheek, and unless saliva crossed the mouth from the left side, we had pure buccal juice. In a few trials, this secretion gave good results, a small amount would activate fistula saliva. A few drops would activate 50 cc. of fistula saliva after several hours incubation and if a large enough quantity was used, it would digest starch.

Orokinase can also be demonstrated in the mixed saliva of man and horse. To demonstrate this action with mixed horse saliva, the saliva obtained from an esophageal fistula should be diluted 1 to 10 or 15, with distilled water and two drops of such a mixture used. Two drops of this mixture will not digest starch, at least within a period of two hours incubation, but when added to 1 cc. of fistula saliva, good digestion results. Table 2 shows in detail one experiment demonstrating the activating properties of two drops of a mixture of mixed horse saliva, 1 to 10 in distilled water.

To demonstrate orokinase in human saliva, the human saliva must be diluted 1 to 50 in distilled water. Two drops of this mixture will be inactive or very slightly active (varying with individuals) but when added to 1 cc. of horse fistula saliva the resulting mixture gives good digestion. Table 3 shows in detail one experiment demonstrating the activating properties of two drops of a mixture of human saliva, 1 to 50 in distilled water.

[2] Fistula saliva is clear but not viscid, and flows like water; buccal juice is clear and very viscid; mixed saliva is clear and viscid but not as viscid as buccal secretion.

TABLE 2

Details of one experiment demonstrating orokinase in mixed horse saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Slight	Clearer	Clearer	Clear	Clear	Blue	Blue	Blue	Blue	Blue	No reduction	Very slight	Slight	Fair	Good
B_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; mixed horse saliva (1–10), 2 drops; starch, 5 cc.
B_s contains: Distilled water, 1 cc.; mixed horse saliva (1–10), 2 drops; starch, 5 cc.
C_s contains: Fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

TABLE 3

Details of one experiment demonstrating orokinase in mixed human saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Quite clear	Clearer	Clearer	Clear	Clear	Blue	Blue	Blue	Blue	Blue	Slight	Good	Heavy	Heavy	Heavy
B_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Fistula saliva, 1 cc.; human saliva (1–50), 2 drops; starch, 5 cc.
B_s contains: Distilled water, 1 cc.; human saliva (1–50), 2 drops; starch, 5 cc.
C_s contains: Fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

When a few drops of mixed horse saliva are added to 50 cc. of fistula saliva, and the mixture incubated for several hours, there seems to be greater digestion than in a fresh mixture of the same amounts. In the few tests we have made with human saliva, we have failed to demonstrate this property.

All attempts to artificially activate horse fistula saliva or the gland extracts have failed. Magnesium salts failed in our experience. Calcium chloride probably does not, or if it does, its action is very slight. Various degrees of acidity and alkalinity, using various acids and bases have also failed. Letting saliva evaporate at room temperature, and using a water solution of the precipitate, failed. Injecting fistula saliva into the mouth failed to activate the fistula saliva. We could not demonstrate orokinase in human saliva, which had been slowly heated in a water bath until the ptyalin had been destroyed. The heating probably killed the orokinase as well as the ptyalin.

Inactive horse saliva very likely becomes self-active with age. Glycerine extracts (50 per cent) of the salivary glands were kept in the ice box and tests made every few days until putrefactive changes set in. We were unable to demonstrate activity except in the case of sublingual extract. This became active at forty days. There was no evidence of putrefaction at this time. Fistula saliva covered with toluol was kept at incubator temperature and no activation had occurred at sixty days.

There are a number of points concerning orokinase which remain to be worked out, and our studies are being continued. A few experiments with the pig strongly point to a similar phenomena.

II. BACTERIA OF THE MOUTH AS ACTIVATING AGENTS

Ellenberger and Hofmeister (4) apparently did not associate the glands of the mouth with the process of saliva activation, but suggested the bacterial flora of the mouth as being the activating agents. In five experiments using cultures from five different horses, we failed to demonstrate activation.

The bacteria were obtained from the oral cavity by means of a sterile swab and grown on plain agar for thirty-six hours at 37.5°C. The growth was washed off with sterile water into a sterile bottle. No attempt was made to differentiate between the various bacteria, and the entire growth was used. Various amounts of the bacterial emulsion were added to 1 cc. of parotid fistula saliva and salivary gland ex-

tracts. To this was added 5 cc. of a 1 per cent starch solution, and the mixture incubated at 40°C. Digestion was studied by noting changes in the viscosity, the color with iodine and the presence of a reducing sugar.

The results obtained were negative in the five experiments and we can conclude from this work that bacteria of the mouth do not possess the property of activating the inactive horse saliva which is emptied into the mouth. If positive results had been obtained in these studies, it was our intention to isolate the various organisms found in the mouth, and attempt to determine the relative activity of the different bacteria.

III. AMYLOLYTIC ACTION OF MIXED SALIVA

Investigators differ in their opinion regarding the presence of amylolytic enzymes in the saliva of the horse. Ellenberger (5) is of the opinion that mixed saliva has strong amylolytic properties. Mills (6) states that horse saliva has a very feeble action upon starches. R. Meade Smith (7) found that horse saliva would convert crushed raw starch to sugar in fifteen minutes, and that mixed saliva had a more marked amylolytic power than individual secretions. Fred Smith (8) states that according to his observations on the horse, saliva has no chemical action on the raw starch of its food.

We have demonstrated in seventeen horses that mixed saliva possesses amylolytic properties, and that when a good sample can be obtained this amylolytic action is about as powerful as human saliva.

Methods of collecting saliva. As a rule it is difficult to stimulate salivary secretion in the horse. A few horses are found, which are habitual slobberers and from which a considerable quantity of saliva can be obtained without much difficulty. We have been unable to find a satisfactory chemical stimulant. Pilocarpine will stimulate a profuse flow of saliva, but the drug itself will digest starch and enough of it will be excreted in the saliva to demonstrate this action. This fact was shown to be the case by Palmer (9) in his studies on ox saliva, and in repeating this work with pilocarpine, we have been able to confirm his statements. In one horse, possessing a parotid duct fistula, 0.5 grain of pilocarpine hydrochloride was administered subcutaneously. A pronounced flow of saliva occurred in about ten minutes and this saliva showed marked amylolytic properties. Fistula saliva from this horse had been previously tested many times when secretion had been stimulated by feeding oats, and was always found to be negative.

In some cases we were able to stimulate a fair amount of secretion by irrigating the mouth with dilute acetic acid, and giving inhalations of strong acetic acid. Teasing with grains or forage, or placing a bit in the mouth also assisted in some cases. In two horses saliva was being secreted, at the time of collecting the sample, and these cases gave good results.

When secretion was stimulated, it was necessary to collect the saliva by means of a spoon, as the animal would swallow as soon as a small quantity collected in the mouth. In the majority of cases only 5 to 10 cc. of saliva could be obtained, and in some animals it was very difficult to collect. In the cases in which it was very difficult to collect the saliva, the small amount of material collected seemed to be a mixture of buccal secretion and mucus, and these samples invariably gave poor results. There was a direct relation between the ease with which the saliva was collected and the amount of digestion. When it was difficult to stimulate and only a small quantity of mouth secretion was obtained, the results were either negative or very poor. This fact in our opinion very largely explains or accounts for the negative or poor results reported by some workers. We obtained negative results in a number of horses, but if we could procure these same horses at a time when the flow of saliva was easily stimulated, we invariably obtained good digestion.

The collection of the mixed secretions from the esophageal fistula was, of course, attained without any difficulties. Every five to ten minutes the horse would swallow from 5 to 20 cc. of mixed mouth secretions.

Methods of study. The usual methods of noting changes in viscosity, color with iodine and the presence of a reducing sugar were employed. The cooked starch used in our studies consisted of a 1 per cent solution of Dakomin corn starch, and the uncooked starch solution was prepared by grinding whole corn or oats in a mill, adding water, straining through cheese cloth, and using the liquid portion. The tubes containing the mixtures under observation were invariably incubated at 40°C.

Results. In eleven horses a potent secretion has been obtained from the mouth, but in several horses we have failed to demonstrate amylolytic activity in the mouth secretions. We not only failed to obtain positive results in some horses, but even the eleven positive cases gave varying results. The degree of activity was directly proportional to the ease with which we obtained our sample. The following table gives a summary of the relative activity in the eleven positive cases.

TABLE 4

Summary of eleven positive cases showing when changes became pronounced

	CLEARING IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120
Number of positive cases.....	3	4	2	1	1	2	—	4	2	3

Table 5 shows in detail one experiment demonstrating the amylolytic activity of mixed horse saliva, obtained from the mouth. The saliva in this case was obtained from an aged grey mare. The secretion was obtained without much difficulty, and was stimulated by teasing with oats.. Digestion was not as strong as that obtained in some horses, neither was it as weak, and the results in this case are suggestive of what can normally be expected. It will be noticed that clearing was not recorded until fifteen minutes, and that at this time slight reduction had occurred. Digestion was not complete in this case at two hours, as indicated by the blue color with iodine. The amount of digestion in this case was similar to that reported by Meade Smith.

With the exception of one horse, the mixed secretions obtained from esophagus were very powerful. The particular animal which gave the digestion below par was a large grey mare, with a parotid fistula on the right side. The secretion swallowed by this mare differed from that in any of the other horses, and was of a consistency similar to that of egg white. This secretion possessed weak amylolytic action, but had strong activating properties when mixed with the inactive fistula saliva. This secretion was evidently largely composed of buccal and lingual secretions. Masticated corn or oats obtained from the esophageal fistula in this mare, however, contained about as much sugar as in the other cases studied.

In five horses the secretions collected from the esophageal fistulae were of a uniform potency, and were somewhat more viscid than the parotid fistula saliva. Even in the horses with parotid duct fistulae, (on one side) the secretions collected from the esophagus were not like those found in the above grey mare, but were similar to those obtained from the horses possessing only esophageal fistulae. In these five horses the mixed secretions possessed very powerful amylolytic action. The starch solution became clear in one to five minutes, the blue color with iodine disappeared within this time, and at these intervals the reduction was very heavy. Table 6 shows in detail one experiment demonstrating the powerful amylolytic activity of mixed saliva, collected from an esophageal fistula.

TABLE 5

Details of one experiment comparing amylolytic action of mixed horse saliva obtained from the mouth, with that of human saliva

TUBE NO.	CLEARING IN MINUTES					COLOR WITH IODINE IN MINUTES					REDUCTION IN MINUTES				
	15	30	60	90	120	15	30	60	90	120	15	30	60	90	120
A_s	Slight	Slight	Clearer	Clearer	Clear	Blue	Blue	Blue	Blue	Blue	Very slight	Slight	Fair	Good	Much
B_s	Clear	Clear	Clear	Clear	Clear	No color	No color	No color	No color	No color	Heavy	Heavy	Heavy	Heavy	Heavy
C_s	No clearing	No clearing	No clearing	No clearing	No clearing	Blue	Blue	Blue	Blue	Blue	No reduction	No reduction	No reduction	No reduction	No reduction
D					No clearing					Blue					No reduction

A_s contains: Mixed horse saliva, 1 cc.; starch, 5 cc.
B_s contains: Mixed human saliva, 1 cc.; starch, 5 cc.
C_s contains: Horse parotid fistula saliva, 1 cc.; starch, 5 cc.
D contains: Distilled water, 1 cc.; starch, 5 cc.

TABLE 6

Details of one experiment comparing amylolytic action of mixed horse saliva obtained from esophageal fistula with that of human saliva

NO. OF TUBE	CLEARING IN MINUTES				COLOR WITH IODINE IN MINUTES				REDUCTION IN MINUTES			
	3	5	15	30	3	5	15	30	3	5	15	30
A_s	Nearly clear	Clear	Clear	Clear	Blue	Faintly blue	No color	No color	Heavy	Heavy	Heavy	Heavy
B_s	Clear	Clear	Clear	Clear	faintly blue	No color	No color	No color	Heavy	Heavy	Heavy	Heavy
C				No clearing				Blue				No reduction

A_s contains: Horse saliva from esophageal fistula, 1 cc.; starch, 5 cc.
B_s contains: Human saliva, 1 cc.; starch, 5 cc.
C contains: Distilled water, 1 cc.; starch, 5 cc.

In a number of experiments we have studied the amylolytic activity of mixed saliva, obtained from esophageal fistulae, compared to that of human saliva on cooked and uncooked starch. When acting upon cooked starch, the two salivas are of about equal activity. Upon raw corn or oats starch prepared by grinding these grains, adding warm water and straining through cheese cloth, the horse saliva is more powerful than the human. In fact horse saliva seems to attack the raw grains as readily as the cooked. Human saliva would also digest these raw grains to a very marked degree.

IV. AMOUNT OF COMPLETE STARCH DIGESTION IN THE MOUTH

Ellenberger (10) has demonstrated that horse saliva can digest raw starch, by showing the presence of a reducing sugar in the food contents escaping from an esophageal fistula, when the diet is composed of sugar-free starches. He reports that on a diet of corn, the food caught from an esophageal fistula one to three minutes after feeding shows the presence of much reducing sugar.

R. Meade Smith (11) on the other hand states that examination of the substances escaping from an esophageal fistula in the horse fed on starchy food, shows that practically no conversion of starch into sugar occurs in the mouth. Smith, however, does believe that horse saliva possesses amylolytic properties, but that it requires at least fifteen

minutes action before digestion can be demonstrated. For this reason, he argues that it is not to be expected that food coming from an esophageal fistula would contain a reducing sugar. Smith is also of the opinion that the digestion started in the mouth is continued in the stomach, and that this digestion is of considerable importance.

In experiments upon six horses we have been able to confirm Ellenberger's work, and have been able to show that on a diet composed of raw corn or oats, the food escaping from the esophageal fistula shows heavy reduction with Fehling's solution, whereas the grain itself or the horse saliva possesses no reducing properties. The first horse, an aged bay gelding, was fed a handful of corn, which was thoroughly masticated and swallowed within three minutes. The corn was caught from the esophagus into a clean beaker, distilled water added, the mixture strained through cheese cloth, and the liquid portion tested at once with Fehling's solution; following which heavy reduction appeared. A handful of corn from the same ear was ground in a hand mill, water added, the mixture strained through cheese cloth, and the liquid portion tested with Fehling's solution; following which no reduction occurred. Mixed mouth secretions escaping from the fistula were also tested, and they gave negative results. Similar tests were made with oats and wheat. The oats showed heavy reduction, but the wheat very little. The wheat also caused the animal to become choked, causing considerable inconvenience, and we have not repeated this experiment.

In five horses we have attempted to make a quantitative determination of the amount of sugar present in the swallowed food, and thus determine the amount of complete starch digestion in the mouth. The starch in the grains was converted into sugar by the official government method (12), and the percentage of sugar determined by Benedict's method. This gave us the total possible amount of sugar. One hundred grams of whole raw corn or oats was then fed, and caught from the fistula into a suitable vessel. Dilute HCl was added and thoroughly mixed with the food to stop further enzyme action. The mixture was then strained through cheese cloth or run through a force filter and the percentage of sugar determined. A determination was made later, and if the results checked with the first, we knew the enzyme had been destroyed.

The amount of complete starch conversion which had taken place in the mouth was not as great as we had anticipated, and our methods were subject to considerable error. We are inclined to think, however,

that our percentages are too low rather than too high. A point worthy of mention is the fact that our horses were all old animals, and they did not masticate the grain as well as young horses and considerable grain was retained in the mouth, which was difficult to remove or estimate the amount. Old horses with poor teeth usually allow some food to remain in the cheeks, and to guard against this, the animals were watered before and after each feed, but this would not remove all of the grain.

Experiments upon these five horses show that approximately 0.5 to 1 per cent of starch in the corn, and 1 to 2 per cent of the oats starch was completely digested in the mouth. Table 7 shows the results of this work.

TABLE 7

Amount of complete starch digestion in the mouth

ANIMAL	TOTAL POSSIBLE PERCENT		PERCENTAGE OF COMPLETE DIGESTION IN MOUTH			REMARKS
	Corn	Oats	Corn	Oats	Wheat	
Bay Gelding..	59.89	49.26	Not estimated, heavy reduction	Not estimated, heavy reduction	Very slight reduction	Good mastication
Black Gelding	59.89	49.26	0.41	1.2	Not estimated	Poor mastication
Bronco Pony	59.89	49.26	0.56	2.7	Not estimated	Poor mastication
Grey Mare....	59.89	49.26	0.58	1.1	Not estimated	Poor mastication
Roan Gelding	59.89	49.26	1.04	1.3	Not estimated	Fair mastication
Sorrel Mare...	59.89	49.26	0.47	0.65	Not estimated	Very poor mastication

In three horses a number of additional tests were made whereby we did not add the acid to the food escaping from the fistula, but allowed the digestion to continue for several hours at incubator temperature. When tested, it was found that on an average 9.7, 13.4 and 6.38 per cent of the corn starch, and 8.4, 12 and 4 per cent of the oats starch had been digested in the three horses respectively. (The third animal had very poor teeth and mastication was very imperfect.)

It is known that corn contains 55 to 60 per cent and oats about 50 per cent of digestible carbohydrates, and our estimations checked well

with these, and of this amount the above amounts were digested after several hours. But here again these figures may not be truly representative of the amounts digested, because it is a well established fact that the products of enzyme activity will stop the action of the enzyme, and this was certainly the case in our experiments. It is also possible that salivary amylase will attack only certain of the carbohydrates in the grains.

SUMMARY

1. Saliva obtained from the parotid ducts or extracts of the salivary glands will not digest starch.

2. It is difficult to stimulate secretion and collect mixed saliva from the mouth of the horse.

3. Saliva collected from the mouth is seldom, if ever, as powerful as that obtained from an esophageal fistula.

4. Mixed mouth secretions obtained from an esophageal fistula have a very powerful amylolytic action.

5. The amylolytic action of mixed horse saliva is equal to that of human saliva on cooked starches, and greater than that of human saliva when acting on raw starches.

6. Mixed horse saliva attacks raw starch as readily as cooked starch.

7. The inactive saliva secreted by the salivary glands is activated in the mouth by the secretions of the glands in the mouth.

8. The name orokinase has been proposed for the activating enzyme found in the mouth secretions.

9. Orokinase can be demonstrated in the mixed mouth secretions of the man and horse.

10. Attempts to artificially activate fistula saliva or gland extracts have failed, but the gland extracts become self-active with age.

11. On a diet of raw corn and oats, food caught from an esophageal fistula a few minutes after feeding, shows the presence of considerable reducing sugar.

12. Salivary digestion started in the mouth is very likely continued in the stomach, and this digestion is more important in the horse than most investigators have been lead to believe.

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AN APPLICATION OF BOYLE'S LAW TO PULSE WAVES IN CLINICAL MEASUREMENT OF BLOOD PRESSURE

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In a recent paper by Erlanger (1) dealing with some conditions occurring in the estimation of blood pressure by the indirect or armlet method, some conclusions are reached which seem incorrect in that they are reached apparently without due regard for the physical laws which are involved. An experimental analysis of the statements made by him has led me to quite opposite conclusions which are presented in the present paper.

The conditions underlying the first deduction which Erlanger made and stated are: An inextensible artery which is inelastic but is easily collapsible and is subjected to diastolic and systolic pressure within, and also subject to pressure without, the artery being surrounded by and placed in a so-called "compression chamber" which chamber is filled with an incompressible fluid which is connected with a manometer whose indicator is moved by a minimum translocation of fluid in the chamber. The pressure outside the artery and in the chamber can be applied to the artery at any desired phase or level; for example, when the inside pressure is at diastolic, or at systolic, or at any level between these two (2).

It is noted here that Erlanger first postulates a chamber with an incompressible fluid and at the same time connected with a manometer which permits movement of the fluid. These two conditions are not in agreement.

Erlanger's deductions are: If a pressure now equal to the diastolic be applied during the diastolic phase in the artery, no oscillations will be produced in the manometer during the pulsations of inside arterial pressure. For, he argues, if the inside pressure rises above the diastolic, the vessel is already completely filled, and being inextensible, cannot expand further and therefore cannot transmit the increase of inside or arterial pressure when it rises above the diastolic level. But

I wish here to point out that if the pressure in the chamber is at the diastolic level and the pressure within the artery is also just at the diastolic level, then it does not at all follow that the artery must necessarily be filled with fluid. Since the artery is readily collapsible (though not elastic) it may be only partly filled, or it may be entirely flat and empty. It may be in any degree of fullness or emptiness. But one must know the amount of fluid within the artery before he can tell whether a rise in arterial pressure will be transmitted to the chamber. As a matter of fact, not unless the artery is completely filled with fluid at the diastolic pressure and the chamber pressure just equal to it is applied without allowing the artery to collapse the slightest amount, can the result obtained by Erlanger be possible.

I have devised an improved apparatus giving more nearly the postulated conditions and also embodying the features outlined by Erlanger. The apparatus is shown in figure 1. My modification eliminates the tambour used by Erlanger, which is a desirable change since the use of the tambour introduces an elastic membrane into the system which is contrary to the desired theoretical condition. In figure 1, *A* is an inelastic artery; *B*, a rigid chamber surrounding the artery filled with water; *C* is a minute capillary tubular air space to show pressure conditions in the chamber. (It is true that theoretically, as with Erlanger's tambour, I have introduced the objectionable elastic substance into the system; but the advantage of the capillary tube of air is that its volume is so minute that the translocation of fluid is practically negligible.) *D* is a valve through which the desired amount of air can be forced into the small capillary tube.

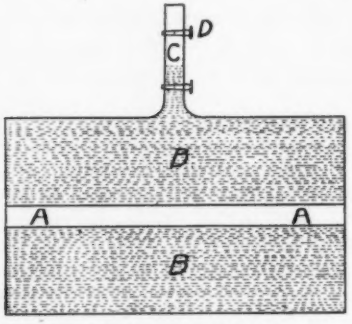

Figure 1

Another statement made by Erlanger in this same paper (3) deals with the same inelastic but collapsible artery within the same compression chamber but filled with an elastic medium, namely air. His conclusion is, in effect, that with the conditions just stated and with a given pulsatory volume change of the artery, the transmitted oscillations are proportional to the initial chamber pressure. For example he gives in figure 2, (4) a diagram which would indicate that if a pres-

sure of say 100 mm. were in the air-filled chamber, and then the artery was expanded say 1 cc. of volume, the pulsatory wave transmitted to the tambour (or other manometer) would show an oscillation of say X mm. Now repeat the observation with the chamber pressure doubled (or raised to 200 mm.) but with the same volume of expansion of the artery which is 1 cc. According to Erlanger there would now be double the size of oscillations recorded by the chamber tambour or 2 X mm.

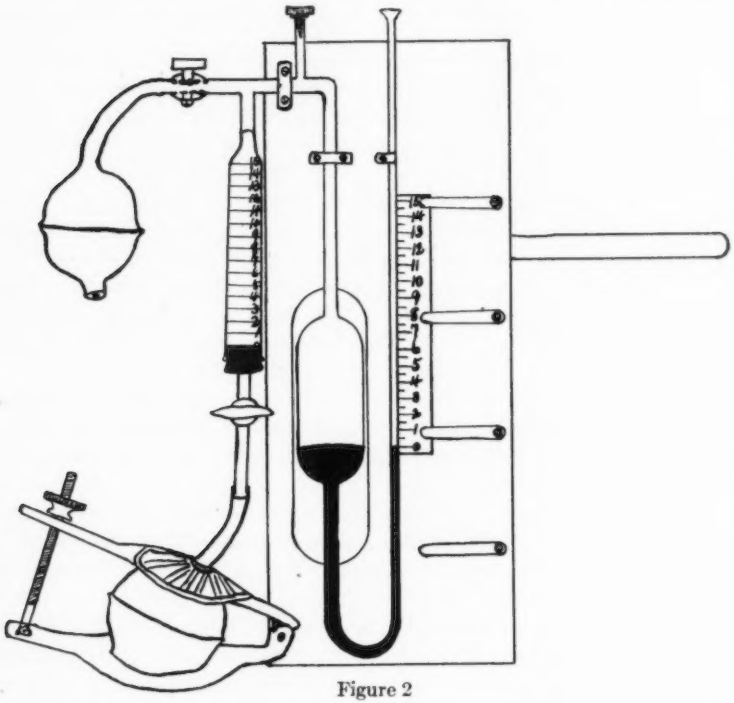

Figure 2

So Erlanger in his diagram shows the transmitted oscillations growing in size as the chamber pressure is increased; beginning at slightly above the diastolic they increase until they are almost doubled when the chamber pressure has reached a point just below systolic level.

But as a matter of fact they do not behave in this way at all. *The fallacy in Erlanger's hypothesis lies in the fact that he regarded only the manometric pressure of the chamber, whereas he should have regarded the*

absolute pressure. The absolute pressure is of course the manometric pressure plus the barometric pressure. In other words the oscillations obtained by varying pressures are in the ratio of $\frac{P}{P'}$, where P is the absolute pressure (or the sum of the manometric and the barometric pressures) and P' is the absolute pressure caused by the compression. $P' - P$ = the oscillations. Boyle's law is $PV = K$, where P is pressure, V the volume, and K a constant, $\frac{K}{V'} = P'$ where V' is the volume produced by adding an incompressible fluid to the air chamber, and P' is the new pressure produced by this addition. A concrete case may be taken. With the air chamber set at 100 cc. capacity, 1 cc. of fluid was forced in by compressing the air to 99 cc. volume. Beginning with a barometric pressure of 747 mm. and zero manometric pressure, the theoretical rise caused by the above experiment is 7.54 mm. of mercury. Beginning with the manometric pressure in the chamber of 50 mm. which is a total or absolute pressure of 797 mm., the oscillation expected as shown by theoretical calculation is 8.05 mm. Beginning with a chamber pressure of 100 mm. the oscillation expected theoretically is 8.55 mm., etc. The theoretical ratio of the oscillation here to the absolute pressure at the beginning is 0.0101 : 1.

The ratio of the size of oscillation at 50 mm. beginning pressure, as compared with the size of oscillations at a beginning pressure of twice that amount, or 100 mm., is 8.05 : 8.55 or 1 : 1.06 plus (instead of 1 : 2 as per Erlanger hypothesis).

The ratio of the size of the oscillation at 0 mm. beginning pressure, as compared with the size of oscillations at a beginning pressure of 100 mm. was 7.54 : 8.55 or 1 : 1.13, instead of 1 : *infinity* which is absurd! (as demanded by Erlanger's hypothesis).

The apparatus shown in figure 2 was used to make these experiments. It consists of a cylinder holding 100 cc. connected on one side with a manometer and provided on the other side with a bulb for forcing in the desired amount of fluid (1 cc. or more). A compression bulb gave the desired initial pressures when these were above the atmospheric or barometric pressure.

The table gives the data; first as obtained from theoretical calculation, second from actual readings as obtained from one of a large series of experiments.

The accord of the two is as close as we expected. Volume of air used, 100 cc. Volume of displacement by compression, 1 cc. Barometric pressure on the day of the experiment, 747 mm.

BAROMETRIC PRESSURE		BEGINNING MANOMETRIC PRESSURE		ABSOLUTE PRESSURE	THEORETICAL OSCILLATIONS CALCULATED	EXPERIMENTAL OSCILLATIONS FOUND	RATIO	
							Theoretical	Experimental
mm.		*mm.*		*mm.*	*mm.*	*mm.*		
747	+	0	=	747	7.54	7.5	1.01	1.004
747	+	50	=	797	8.05	8.0	1.01	1.004
747	+	100	=	847	8.55	9.0	1.01	1.062
747	+	150	=	897	9.06	9.0	1.01	1.062

$$PV = K \quad P'V' = K$$

$$\frac{K}{V'} = P' \quad P' - P = \text{size of oscillation.}$$

$$\frac{\text{Absolute pressure P}}{\text{Absolute pressure P}'} = \text{Ratio of oscillation}$$

It is generally known that the volume of the compression chamber determines the relation between the magnitude of oscillations and arterial pressure. It may be remembered here that this relation is a simple numerical one, viz., a chamber of twice the volume will give an oscillation of one-half the magnitude with a given pulsatory arterial volume change.

CONCLUSIONS

A simple apparatus is described for helping to show the workings of certain physical laws regarding the indirect transmission of arterial pulse waves such as are used in clinical measurement of blood pressure.

Erlanger states that with an inelastic but collapsible arterial segment surrounded with a rigid chamber and the chamber filled with incompressible fluid (water) when the arterial (inside) pressure is at diastolic, and the chamber (outside) pressure is closed at exactly the diastolic level, no pressure waves can be transmitted to the chamber from the artery. I find that this is not true except where the artery at the closing of the chamber is fully distended with fluid, for when the arterial segment is only partially filled, the pressure waves of the artery are wholly transmitted to the chamber (excepting only when the volume occupied by the translocation of fluid in the filling of the artery is less than the compression volume of the tambour, manometer or capillary tube caused by the pulsatory change of arterial pressure).

Erlanger also states that the oscillations of pressure in the air filled arm band or air filled compression chamber, with a given volume pulse change would be proportional to the manometric pressure of the arm

band. This does not hold. On the contrary I find, upon testing this hypothesis with the help of the physical apparatus described above, that the oscillations of volume occupied by a given mass of gas produces inversely proportional oscillations of *absolute pressure*. Or in other words, *the absolute pressure of a given mass of gas is inversely proportional to its volume*. In short, it is another way of stating Boyle's law that the absolute pressure of a gas is proportional to the concentration of the gas.

Therefore the results of the present work are in harmony with Boyle's law, but are contrary to Erlanger's hypothesis.

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